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(54) GENE CONCERNING BRASSINOSTEROID-SENSITIVITY OF PLANTS AND UTILIZATION THEREOF

(57) The present inventors successfully produced rice dwarf mutant *d61* and also isolated the *OsBRI1* gene which corresponds to a region in the *d61* locus. *OsBRI1* is found to increase plant brassinosteroid sensitivity. Moreover, the present inventors showed that OsBRI1 functions in growth and development process of- rice, such as, internode elongation by inducing internode cell elongation and the inclination of the lamina joint. By introducing antisense nucleotides or dominant negative of *OsBRI1*, the present inventors produced transgenic rice plants whose phenotype was modified.

EP 1 275 719 A1

Description

Technical Field

[0001] The present invention relates to a novel gene involved in plant brassinosteroid sensitivity, the protein encoded by the gene, and production and use of the same.

Background Art

[0002] Research in plant molecular biology has advanced dramatically in recent years and is necessary for the analysis of various physiological phenomena. Dwarfism caused by artificial modification of grass type, especially the control of elongation growth, prevents plants from lodging due to overgrowth caused by over fertilization. This prevention of lodging was demonstrated in Mexican wheat during the "Green Revolution" and in miracle rice (IR-8) developed by the International Rice Research Center. Furthermore, in the case of cultivation at high density, such as rice cultivation, yields are expected to increase as a result of the increase in the amount of sun light each plant receives due to the formation of upright leaves. Moreover, these modifications are very important breeding targets because they may result in yield increases and also increase the efficiency of plant growth maintenance. However, current breeding methods cannot artificially modify plant morphology.

[0003] Dwarfism is an abnormal growth caused by mutation in genes involved in controlling normal elongation growth. Plant elongation growth is the result of accumulation of cell division and cell elongation. Cell division and cell elongation are controlled by complex effects caused by various factors, such as, exogenous environmental factors including temperature and light and endogenous environmental factors including plant hormones. Therefore, it is predicted that many genes, such as those related to plant hormone biosynthesis and hormone receptors directly and those related to the control of the expression of these genes, are involved in the dwarfism (Sakamoto et al. (2000) Kagaku to Seibutsu, 38: 131-139).

[0004] Almost all modern cultivars of *japonica* rice develop 15-16 phytomers, consisting of leaves, axillary buds, and short or elongated internodes, during the vegetative stage. After the shoot meristem shifts from the vegetative to the reproductive phase, the reproductive meristem develops about 10 phytomers consisting of undeveloped leaf, an elongated internode, and an axillary which develops into the primary rachis branch. The phytomers formed in the vegetative stage can be classified into three types in terms of the morphology of the internode (Suetsugu, Isao. (1968) Japan. J. Crop Sci. 37, 489-498). The first type is developed in the juvenile phase and form undifferentiated nodes and internodes. After the shoot apical meristem (SAM) shifts from the juvenile to the adult phase, the nodal plate of the second type differentiates and the central part of the internode thereof decays to produce an air space. The third type contains long elongated internodes as a result of growth from the intercalary meristem.

[0005] Phytomers of type 1 are produced first during vegetative development, followed by type 2 and then type 3 phytomers. Under normal growth conditions, the number of phytomers of each type in many *japonica* cultivars is 4- 5, 6-7, and 4- 5. The transition from type 1 to type 2 is strictly regulated. After the serial development of 4- 5 type 1 phytomers, depending on the cultivar, the SAM develops type 2 phytomers. However, the transition from type 2 to type 3 does not depend on the number of development of type 2 phytomers. The SAM develops 15- 16 phytomers and shifts from the vegetative to the reproductive stage, the type 3 phytomers then start to develop, and the uppermost four or five internodes thereof start to elongate. If the timing of the transition is changed by unusual growth conditions, the number of the type 2 phytomers always affected thereby, but the number of the type 3 phytomer with elongated internode is unchanged (Suetsugu, isao. (1968) Japan. J. Crop Sci. 37, 489-498). This indicates that the transition of the SAM from the vegetative to the reproductive phase in rice induces internode elongation, as well as in *Arabidopsis*.

[0006] However, there is an important difference between rice and *Arabidopsis*. The elongated internodes in rice are derived from the vegetative SAM while those in *Arabidopsis* come from the reproductive SAM. In rice, the uppermost four or five internodes develop from the vegetative SAM and initially are indistinguishable from the lower type 2 internodes. When the SAM shifts to the reproductive phase, differentiation into type 3 internodes occurs due to the development of intercalary meristems in the internodes. This synchronicity between the phase change of the SAM and the development of the intercalary meristem leads to the possibility that these processes might be linked by a signal coming from the SAM to the uppermost four or five phytomers when its phase change occurs.

[0007] A large number of dwarf mutants of rice have been collected and characterized because of their agronomic importance. These dwarf mutants are categorized into six groups based on the elongation pattern of the upper four to five internodes (Fig. 1; redrawn from Takeda, K. (1974) Bull. Fac. Agr. Hirosaki Univ. 22, 19-30. In rice, each internode is numbered from top to bottom such that the uppermost internode just below the panicle is first). The present inventors can see that in the dn-type mutants the length of each internode is almost uniformly reduced, resulting in an elongation pattern similar to that of the wild type plant. In contrast, the dm-type mutants show specific reduction of the second internode. Similar shortening of a specific internode is also observed in the sh- and d6-type mutants, in which only the

uppermost first internode or internodes below the uppermost are shortened, respectively. As these mutants with specifically shortened internodes, such as the dm-, d6-, and sh-types, might be defective in the perception of signals coming from the SAM, they should be especially useful for the study of the mechanism of internode elongation and its relationship to changes in the SAM.

[0008] Brassinosteroids (BRs) are plant growth-promoting natural products that are required for plant growth and development. There are only a few reports on the physiological effects of brassinosteroids in the growth and development of rice and other plants of the *Gramineae* family. Physiological researches indicate that exogenous brassinosteroids alone, or in combination with auxin, enhance bending of the lamina joint in rice. The lamina joint has been used for a sensitive bioassay of brassinosteroids (Maeda, E. (1965) Physiol. Plant. 18, 813-827; Wada, K. et al. (1981) Plant and Cell Physiol. 22, 323-325; Takeno, K. and Pharis, R. P. (1982) Plant Cell Physiol. 23, 1275-1281), because of high sensitivity thereof to brassinosteroids. In etiolated wheat seedlings treatment with brassinolide or its derivative, castasterone, stimulates unrolling of the leaf blades (Wada, K. et al. (1985) Agric. Biol. Chem. 49, 2249-2251). Treatment with low or high concentrations of brassinosteroids promotes or inhibits the growth of roots in rice, respectively (Radi, S. H. and Maeda, E. (1988) J. Crop Sci. 57, 191-198). Brassinosteroids also promote the germination of rice seeds (Yamaguchi, T. et al. (1987) Stimulation of germination in aged rice seeds by pre-treatment with brassinolide. In: Proceeding of the fourteenth annual plant growth regulator society of America Meeting Honolulu. (Cooke AR), pp. 26-27).

[0009] Although these results indicate only effects due to exogenous brassinosteroids, not due to endogenous brassinosteroids, they do suggest that endogenous brassinosteroids have an important role in growth and developmental processes in plants of the *Gramineae* family.

[0010] On the other hand, there is some apparent disagreement in the literature as to whether brassinosteroids induce cell elongation in plants of the *Gramineae* family. That is, brassinolide treatment does not induce elongation of the leaf sheath of rice (Yokota, T. and Takahashi, N. (1986) Chemistry, physiology and agricultural application of brassinolide and related steroids. In: Plant growth substances 1985. (Bopp M, Springer-Verlag, Berlin/Heidelberg/New York) pp.129-138), but it does induce elongation of the coleoptile and mesocotyl in maize (He, R. -Y. et al. (1991) Effects of brassinolide on growth and chilling resistance of maize seedlings. In: Brassinosteroids-Chemistry, Bioactivity and Applications ACS symposium series 474. (Cutler HGC, Yokota T, Adam G, American Chemical Society, Washington DC), pp. 220-230).

[0011] As shown by brassinosteroids synthesis mutants or brassinosteroids insensitive mutants that show severe dwarfism with abnormal development of organs, the function of brassinolide is known in dicotyledonous plants.

[0012] However, little is known about the function of endogenous brassinosteroids in monocotyledonous plants, such as rice or other plants of the *Gramineae* family.

Disclosure of the Invention

[0013] The object of the present invention is to provide novel genes involved in brassinosteroid sensitivity from plants, preferably from monocotyledonous plants. Another object of the present invention is to modify plant brassinosteroid sensitivity by controlling the expression of the gene. The modification in plant brassinosteroid sensitivity causes a change in plant morphology. The preferable embodiment of the present invention provides plants with erect leaves which become dwarfed due to the suppression of internode elongation caused by decreased brassinosteroid sensitivity.

[0014] By treatment with mutagenesis agent, the present inventors isolated a novel rice dwarf mutant strain *d61* (*d61-1* and *d61-2*) which showed lower brassinosteroid sensitivity and had shorter internodes than wild type plants.

[0015] Linkage analysis indicated that the *d61* locus was highly linked to a gene region that was homologous to Arabidopsis BRI1. The present inventors isolated the gene (*OsBRI1*), which was homologous to Arabidopsis *BRI1* gene, by screening of a rice genomic DNA library. Nucleotide sequence analysis of the *OsBRI1* gene from *d61-1* and *d61-2* mutants indicated that there were single nucleotide substitutions causing amino acid substitutions at different sites in each *d61* allele.

[0016] Moreover, in order to confirm that the *OsBRI1* gene corresponds to the *d61* locus, the *OsBRI1* gene was introduced into *d61* mutants. As a result, the *OsBRI1* gene complimented the *d61* phenotype and caused the mutant strain to have a wild-type phenotype. Therefore, it was indicated that *d61* mutants are caused by loss of function of the *OsBRI1* gene. Phenotypic analysis of plants revealed that the *OsBRI1* gene functions in various growth and development processes of rice including internode elongation caused by formation of intercalary meristem and induction of internode cell longitudinal elongation, inclination of the lamina joint, and skotomorphogenesis in the dark.

[0017] Moreover, in the case where transgenic rice plants with *OsBRI1* antisense nucleotide were produced, most transgenic plants produced erect leaves during seedling growth. All of the transgenic plants showed dwarf phenotype of various levels. Plants transformed with *OSBRI1* having the dominant negative phenotype showed the same result.

[0018] The present invention had been made in view of such findings, and relates to a novel gene involved in plant brassinosteroid sensitivity, the protein encoded by the gene, and production and use of the same. Moreover, the present invention relates to the production of modified plant by controlling expression of the gene.

[0019] More specifically, this invention provides:

(1) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;

(2) the DNA of (1), wherein the DNA is a cDNA or a genomic DNA;

(3) the DNA of (1), wherein the DNA comprises a coding region of the nucleotide sequence of SEQ ID NO: 1 or 3;

(4) a DNA encoding a protein which has 55% or more homology to the amino acid sequence of SEQ ID NO: 2 and which is functionally equivalent to a protein comprising the amino acid sequence of SEQ ID NO: 2, the DNA being selected from the group consisting of

(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2 in which one or more amino acids are substituted, deleted, added, and/or inserted; and
(b) a DNA hybridizing under stringent conditions with a DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3;

(5) the DNA of (4), wherein the DNA encodes a protein having a function selected from the group consisting of a function of increasing brassinosteroid sensitivity in a plant, a function of inducing elongation of internode cells of a stem of a plant, a function of positioning microtubules perpendicular to the direction of elongation in an internode of a stem of a plant, a function of suppressing elongation of an internode of a neck of a plant, and a function of increasing inclination of a lamina of a plant;

(6) the DNA of (4) or (5), wherein the DNA is derived from a monocotyledonous plant;

(7) the DNA of (6), wherein the DNA is derived from a plant of the Gramineae family;

(8) a DNA encoding an antisense RNA complementary to a transcript of the DNA of any one of (1) to (7);

(9) a DNA encoding an RNA having ribozyme activity which specifically cleaves a transcript of the DNA of any one of (1) to (7);

(10) a DNA which encodes an RNA repressing expression of the DNA of any one of (1) to (7) due to co-suppression when expressed in a plant cell and which has 90% or more homology to the DNA of any one of (1) to (7);

(11) a DNA which encodes a protein having a dominant negative phenotype to that of a protein encoded by the DNA of any one of (1) to (7);

(12) a vector which comprises the DNA of any one of (1) to (7);

(13) a transformed cell which comprises the DNA of any one of (1) to (7) or the vector of (12);

(14) a protein encoded by the DNA of any one of (1) to (7);

(15) a method for producing the protein of (14), the method comprising the steps of culturing the transformed cell of (13) and recovering an expressed protein from the transformed cell or a culture supernatant thereof;

(16) a vector comprising the DNA of any one of (8) to (11);

(17) a transformed plant cell comprising the DNA of any one of (1) to (11) or the vector of (12) or (16);

(18) a transformed plant comprising the transformed plant cell of (17);

(19) a transformed plant which is a progeny or a clone of the transformed plant of (18);

(20) a breeding material of the transformed plant of (18) or (19); and

(21) an antibody which binds to the protein of (14).

[0020] The present invention provides a DNA encoding the OsBRI1 protein derived from rice. The nucleotide sequence of *OsBRI1* cDNA is shown in SEQ ID NO: 1, the amino acid sequence of the protein encoded by the DNA is shown in SEQ ID NO: 2, and the nucleotide sequence of the genomic DNA of *OsBRI1* is shown in SEQ ID NO: 3 (the genomic DNA of SEQ ID NO: 3 consists of one exon with no intron).

[0021] The gene of the present invention causes a rice dwarf mutant (*d61*) which has short internodes and reduced brassinosteroid sensitivity compared to the wild type. Therefore, it is possible to modify plant morphology by controlling the expression of the *OsBRI1* gene.

[0022] The preferable modification in plant morphology in the present invention includes dwarfism of plants by suppressing expression of the DNA of the present invention. Dwarfism of plants has great value in agriculture and horticulture. For example, reduction of height of plants can reduce the tendency of plants to lodge and can thereby increase seed weights. Moreover, it is possible to increase the number of plant individuals which can be planted per unit area by reducing height of plants and by making plant shape per plant more compact. These plant modifications have great value specifically in the production of crops such as rice, corn, wheat, and such. It is also possible to produce ornamental plants with new aesthetic value by dwarfism of height or culm length of plants. It is also possible to produce miniature vegetables or fruits with new commercial value, such as "bite-size", by dwarfism of them. Other than for industrial plants, dwarfism is important for experimental plants because, for example, dwarf plants are not only more easily handled but they also help utilize experimental space more effectively by decreasing cultivation space.

[0023] It is possible to consider that brassinosteroid sensitivity can be increased in brassinosteroid low sensitive plants by expressing the DNA of the present invention in the plants. Thereby, the yield of whole plants may be increased by growing taller plants. Thus, this will be especially useful for increasing yield for whole feed crops.

[0024] DNA encoding the OsBRI1 protein of the present invention includes genomic DNA, cDNA, and chemically synthesized DNA. A genomic DNA and cDNA can be prepared according to conventional methods known to those skilled in the art. More specifically, a genomic DNA can be prepared, for example, as follows: (1) extract genomic DNA from plant cells or tissues; (2) construct a genomic library (utilizing a vector, such as plasmid, phage, cosmid, BAC, PAC, and such) ; (3) spread the library; and (4) conduct colony hybridization or plaque hybridization using a probe prepared based on the DNA encoding a protein of the present invention (e.g., SEQ ID NO: 1 or 3). Alternatively, a genomic DNA can be prepared by PCR, using primers specific to a DNA encoding the protein of the present invention (e.g. SEQ ID NO: 1 or 3). On the other hand, cDNA can be prepared, for example, as follows: (1) synthesize cDNAs based on mRNAs extracted from plant cells or tissues; (2) prepare a cDNA library by inserting the synthesized cDNA into vectors, such as λZAP; (3) spread the cDNA library; and (4) conduct colony hybridization or plaque hybridization as described above. Alternatively, cDNA can be also prepared by PCR.

[0025] The present invention includes DNAs encoding proteins functionally equivalent to the OsBRI1 protein of SEQ ID NO: 2 . Herein, the term "functionally equivalent to the OsBRI1 protein" means that the object protein has equal functions to those of the OsBRI1 protein of SEQ ID NO: 2, such as, for example, a function of increasing brassinosteroid sensitivity in a plant, a function of inducing elongation of an internode of a stem of a plant, a function of positioning microtubules perpendicular to the direction of elongation in internode cells of a stem of a plant, a function of suppressing elongation of an internode of a neck of a plant, and/or a function of increasing inclination of a lamina of a plant. Such DNA is derived preferably from monocotyledonous plants, more preferably from plants of the Gramineae family, and most preferably from rice.

[0026] Examples of such DNAs include those encoding mutants, derivatives, alleles, variants, and homologues comprising the amino acid sequence of SEQ ID NO: 2 wherein one or more amino acids are substituted, deleted, added, and/or inserted.

[0027] Examples of methods for preparing a DNA encoding a protein comprising altered amino acids well known to those skilled in the art include the site-directed mutagenesis (Kramer, W. and Fritz, H. -J. (1987) "Oligonucleotide-directed construction of mutagenesis via gapped duplex DNA." Methods in Enzymology, 154: 350-367). The amino acid sequence of a protein may also be mutated in nature due to the mutation of a nucleotide sequence. A DNA encoding proteins having the amino acid sequence of a natural OsBRI1 protein (SEQ ID NO: 2) wherein one or more amino acids are substituted, deleted, and/or added are also included in the DNA of the present invention, so long as they encode a protein functionally equivalent to the natural OsBRI1 protein. Additionally, nucleotide sequence mutants that do not give rise to amino acid sequence changes in the protein (degeneracy mutants) are also included in the DNA of the present invention. The number of nucleotide mutations of the DNA of interest corresponds to, at amino acid level, typically 100 residues or less, preferably 50 residues or less, more preferably 20 residues or less, and still more preferably 10 residues or less (for example, 5 residues or less, or 3 residues or less).

[0028] Whether a certain DNA actually encodes a protein which has a function of increasing inclination of a lamina of a plant can be evaluated, for example, by performing a "lamina joint test" for plants in which the expression of the DNA has been suppressed and by comparing the results with those for wild-type plants (See Example 4). The result of the test may also be an index for evaluating brassinosteroid sensitivity in a plant. In order to evaluate whether the DNA encodes a protein which has a function of inducing elongation of an internode of a stem of a plant, a function of

positioning microtubules perpendicular to the direction of elongation in internode cells of a stem of a plant, or a function of suppressing elongation of an internode of a neck of a plant, the morphology of the internode cell of the plant in which expression of the DNA has been suppressed can be observed to be compared with that of wild type (See Examples 2 and 3).

[0029] A DNA encoding a protein functionally equivalent to the OsBRI1 protein described in SEQ ID NO: 2 can be produced, for example, by methods well known to those skilled in the art including: methods using hybridization techniques (Southern, E.M. (1975) Journal of Molecular Biology, 98, 503); and polymerase chain reaction (PCR) techniques (Saiki, R. K. et al. (1985) Science, 230, 1350-1354; Saiki, R. K. et al. (1988) Science, 239, 487-491). That is, it is routine for a person skilled in the art to isolate a DNA with high homology to the *OsBRI1* gene from rice and other plants using the *OsBRI1* gene (SEQ ID NO: 1 or 3) or parts thereof as a probe, and oligonucleotides hybridizing specifically to the gene as a primer. Such DNA encoding proteins functionally equivalent to the OsBRI1 protein, obtainable by hybridization techniques or PCR techniques, are included in the DNA of this invention.

[0030] Hybridization reactions to isolate such DNAs are preferably conducted under stringent conditions. Stringent hybridization conditions of the present invention include conditions such as: 6 M urea, 0.4% SDS, and 0.5x SSC; and those which yield a similar stringency with the conditions. DNAs with higher homology are expected to be isolated efficiently when hybridization is performed under conditions with higher stringency, for example, 6 M urea, 0.4% SDS, and 0.1x SSC. Those DNAs isolated under such conditions are expected to encode a protein having a high amino acid level homology with OsBRI1 protein (SEQ ID NO: 2). Herein, "high homology" means an identity of at least 55% or more, more preferably 70% or more, and most preferably 90% or more (e.g., 95% or more), between full-length of amino acids.

[0031] The degree of homology of one amino acid sequence or nucleotide sequence to another can be determined by following the algorithm BLAST by Karlin and Altschul (Proc. Natl. Acad. Sci. USA, 90: 5873-5877, 1993). Programs such as BLASTN and BLASTX were developed based on this algorithm (Altschul et al. J. Mol. Biol. 215: 403-410, 1990). To analyze a nucleotide sequences according to BLASTN based on BLAST, the parameters are set, for example, as score= 100 and word length= 12. On the other hand, parameters used for the analysis of amino acid sequences by the BLASTX based on BLAST include, for example, score= 50 and word length= 3. Default parameters of each program are used when using BLAST and Gapped BLAST program. Specific techniques for such analysis are known in the art (http://www.ncbi.nlm.nih.gov.)

[0032] The DNA of the present invention can be used, for example, to prepare recombinant proteins, produce transformed plants with phenotypes altered by controlling expression thereof as described above, and so on.

[0033] A recombinant protein is usually prepared by inserting a DNA encoding a protein of the present invention into an appropriate expression vector, introducing said vector into an appropriate cell, culturing the transformed cells, and purifying expressed proteins. A recombinant protein can be expressed as a fusion protein with other proteins so as to be easily purified, for example, as a fusion protein with maltose binding protein in *Escherichia coli* (New England Biolabs, USA, vector pMAL series), as a fusion protein with glutathione-S-transferase (GST) (Amersham Pharmacia Biotech, vector pGEX series), or tagged with histidine (Novagen, pET series). The host cell is not limited so long as the cell is suitable for expressing the recombinant protein. It is possible to utilize yeasts or various animal, plant, or insect cells besides the above described *E. coli*. A vector can be introduced into a host cell by a variety of methods known to one skilled in the art. For example, a transformation method using calcium ions (Mandel, M. and Higa, A. (1970) Journal of Molecular Biology, 53, 158-162; Hanahan, D. (1983) Journal of Molecular Biology, 166, 557-580) can be used to introduce a vector into *E. coli*. A recombinant protein expressed in host cells can be purified and recovered from the host cells or the culture supernatant thereof by known methods. When a recombinant protein is expressed as a fusion protein with maltose binding protein or other partners, the recombinant protein can be easily purified by affinity chromatography.

[0034] The resulting protein can be used to prepare an antibody that binds to the protein. For example, a polyclonal antibody can be prepared by immunizing immune animals, such as rabbits, with a purified protein of the present invention or its portion, collecting blood after a certain period, and removing clots. A monoclonal antibody can be prepared by fusing myeloma cells with the antibody-forming cells of animals immunized with the above protein or its portion, isolating a monoclonal cell expressing a desired antibody (hybridoma), and recovering the antibody from the cell. The obtained antibody can be utilized to purify or detect a protein of the present invention. Accordingly, the present invention includes antibodies that bind to proteins of the invention.

[0035] In order to produce a transformed plant in which DNAs of the present invention are expressed, a DNA encoding a protein of the present invention is inserted into an appropriate vector; the vector is then introduced into a plant cell; and finally, the resulting transformed plant cell is regenerated.

[0036] On the other hand, a transformed plant with suppressed expression of DNAs of the present invention can be created using DNA that represses the expression of a DNA encoding a protein of the present invention: wherein the DNA is inserted into an appropriate vector, the vector is introduced into a plant cell, and then, the resulting transformed plant cell is regenerated. The phrase "suppression of expression of DNA encoding a protein of the present invention"

includes suppression of gene transcription as well as suppression of translation into protein. It also includes not only the complete inability of expression of DNA but also reduction of expression.

[0037] The expression of a specific endogenous gene in plants can be repressed by methods utilizing antisense technology, the methods which are commonly used in the art. Ecker et al. were the first to demonstrate the antisense effect of an antisense RNA introduced by electroporation in plant cells by using the transient gene expression method (J. R. Ecker and R. W. Davis (1986) Proc. Natl. Acad. Sci. USA 83: 5372). Thereafter, the target gene expression was reportedly reduced in tobacco and petunias by expressing antisense RNAs (A. R. van der Krol et al. (1988) Nature 333: 866). The antisense technique has now been established as a means to repress target gene expression in plants.

[0038] Multiple factors are required for antisense nucleic acid to repress the target gene expression. These include, inhibition of transcription initiation by triple strand formation; suppression of transcription by hybrid formation at the site where the RNA polymerase has formed a local open loop structure; transcription inhibition by hybrid formation with the RNA being synthesized; suppression of splicing by hybrid formation at the junction between an intron and an exon; suppression of splicing by hybrid formation at the site of spliceosome formation; suppression of mRNA translocation from the nucleus to the cytoplasm by hybrid formation with mRNA; suppression of splicing by hybrid formation at the capping site or at the poly A addition site; suppression of translation initiation by hybrid formation at the binding site for the translation initiation factors; suppression of translation by hybrid formation at the site for ribosome binding near the initiation codon; inhibition of peptide chain elongation by hybrid formation in the translated region or at the polysome binding sites of mRNA; and suppression of gene expression by hybrid formation at the sites of interaction between nucleic acids and proteins. These factors repress the target gene expression by inhibiting the process of transcription, splicing, or translation (Hirashima and Inoue, "Shin Seikagaku Jikken Koza (New Biochemistry Experimentation Lectures) 2, Kakusan (Nucleic Acids) IV, Idenshi No Fukusei To Hatsugen (Replication and Expression of Genes)", Nihon Seikagakukai Hen (The Japanese Biochemical Society), Tokyo Kagaku Dozin, pp. 319-347, (1993)).

[0039] An antisense sequence of the present invention can repress the target gene expression by any of the above mechanisms. In one embodiment, if an antisense sequence is designed to be complementary to the untranslated region near the 5' end of the gene's mRNA, it will effectively inhibit translation of a gene. It is also possible to use sequences complementary to the coding regions or to the untranslated region on the 3' side. Thus, the antisense DNA used in the present invention includes DNA having antisense sequences against both the untranslated regions and the translated regions of the gene. The antisense DNA to be used is connected downstream from an appropriate promoter, and, preferably, a sequence containing the transcription termination signal is connected on the 3' side. The DNA thus prepared can be transfected into the desired plant by known methods. The sequence of the antisense DNA is preferably a sequence complementary to the endogenous gene of the plant to be transformed or a part thereof, but it need not be perfectly complementary so long as it can effectively inhibit the gene expression. The transcribed RNA is preferably at least 90%, and most preferably at least 95% complementary to the transcribed products of the target gene. Sequence complementarity may be determined using the above-described search.

[0040] In order to effectively inhibit the expression of the target gene by means of an antisense sequence, the antisense DNA should be at least 15 nucleotides long, preferably at least 100 nucleotides long, and more preferably at least 500 nucleotides long. The antisense DNA to be used is generally shorter than 5 kb, and preferably shorter than 2.5 kb.

[0041] DNA encoding ribozymes can also be used to repress the expression of endogenous genes. A ribozyme is an RNA molecule that has catalytic activity. There are many ribozymes having various activities. Research on ribozymes as RNA cleaving enzymes has enabled the design of a ribozyme that site-specifically cleaves RNA. While some ribozymes of the group I intron type or the M1RNA contained in RNaseP consist of 400 nucleotides or more, others belonging to the hammerhead type or the hairpin type have an activity domain of about 40 nucleotides (Makoto Koizumi and Eiko Ohtsuka, (1990) Tanpakushitsu Kakusan Kohso (Nucleic acid, Protein, and Enzyme), 35: 2191).

[0042] The self-cleavage domain of a hammerhead type ribozyme cleaves at the 3' side of C15 of the sequence G13U14C15. Formation of a nucleotide pair between U14 and A at the ninth position is considered important for the ribozyme activity. Furthermore, it has been shown that the cleavage also occurs when the nucleotide at the 15th position is A or U instead of C (M. Koizumi et al., (1988) FEBS Lett. 228: 225). If the substrate binding site of the ribozyme is designed to be complementary to the RNA sequences adjacent to the target site, one can create a restriction-enzyme-like RNA cleaving ribozyme which recognizes the sequence UC, UU, or UA within the target RNA (M. Koizumi et al., (1988) FEBS Lett. 239: 285; Makoto Koizumi and Eiko Ohtsuka, (1990) Tanpakushitsu Kakusan Kohso (Protein, Nucleic acid, and Enzyme), 35: 2191; M. Koizumi et al., (1989) Nucleic Acids Res. 17: 7059). For example, in the coding region of the *OsBRI1* gene (SEQ ID NO: 1 or 3), there is a plurality of sites that can be used as the ribozyme target.

[0043] The hairpin type ribozyme is also useful in the present invention. A hairpin type ribozyme can be found, for example, in the minus strand of the satellite RNA of tobacco ringspot virus (J. M. Buzayan, Nature 323: 349 (1986)). This ribozyme has also been shown to target-specifically cleave RNA (Y. Kikuchi and N. Sasaki, (1992) Nucleic Acids Res. 19: 6751; Yo Kikuchi, (1992) Kagaku To Seibutsu (Chemistry and Biology) 30: 112).

[0044] The ribozyme designed to cleave the target is fused with a promoter, such as the cauliflower mosaic virus

35S promoter, and with a transcription termination sequence, so that it will be transcribed in plant cells. However, if extra sequences have been added to the 5' end or the 3' end of the transcribed RNA, the ribozyme activity can be lost. In this case, one can place an additional trimming ribozyme, which functions in cis to perform the trimming on the 5' or the 3' side of the ribozyme portion, in order to precisely cut the ribozyme portion from the transcribed RNA containing the ribozyme (K. Taira et al. (1990) Protein Eng. 3: 733; A. M. Dzalanott and J. J. Bujarski (1989) Proc. Natl. Acad. Sci. USA 86: 4823; C. A. Grosshands and R. T. Cech (1991) Nucleic Acids Res. 19: 3875; K. Taira et al. (1991) Nucleic Acid Res. 19: 5125). Multiple sites within the target gene can be cleaved by arranging these structural units in tandem to achieve greater effects (N. Yuyama et al. (1992) Biochem. Biophys. Res. Commun. 186: 1271). By using such ribozymes, it is possible to specifically cleave the transcripts of the target gene in the present invention, thereby repressing the expression of said gene.

[0045] Endogenous gene expression can also be repressed by co-suppression through the transformation by DNA having a sequence identical or similar to the target gene sequence. "Co-suppression" refers to the phenomenon in which, when a gene having a sequence identical or similar to the target endogenous gene sequence is introduced into plants by transformation, expression of both the introduced exogenous gene and the target endogenous gene becomes repressed. Although the detailed mechanism of co-suppression is unknown, it is frequently observed in plants (Curr. Biol. (1996) 7: R793 (1997), Curr. Biol. 6: 810). For example, if one wishes to obtain a plant body in which the *OsBRI1* gene is co-repressed, the plant in question can be transformed with a vector DNA designed so as to express the *OsBRI1* gene or DNA having a similar sequence to select a plant having the *OsBRI1* mutant character, e.g., a plant with suppressed internode elongation, among the resultant plants. The gene to be used for co-suppression does not need to be completely identical to the target gene, but it should have at least 70% or more sequence identity, preferably 80% or more sequence identity, and more preferably 90% or more (e.g., 95% or more) sequence identity. Sequence identity may be determined by above-described search.

[0046] In addition, endogenous gene expression in the present invention can also be repressed by transforming the plant with a gene having the dominant negative phenotype of the target gene. Herein, "a DNA encoding the protein having the dominant negative phenotype" refers to a DNA encoding a protein which, when the DNA is expressed, can eliminate or reduce the activity of the protein encoded by the endogenous gene of the present invention inherent to the plant. Preferably, it is a DNA encoding the peptide (e.g., peptide which contains from 739 to 1035 residues of amino acids of SEQ ID NO: 2 or peptides of another protein equivalent to the peptide) which lacks the N-terminal region but contains the kinase region of the protein of the present invention. Whether the DNA of interest has the function to eliminate or enhance activity of the endogenous gene of the present invention can be determined, as mentioned above, by whether the DNA of interest eliminates or reduces a function of increasing brassinosteroid sensitivity in a plant, a function of inducing elongation of an internode of a stem of a plant, a function of positioning microtubules perpendicular to the direction of elongation in internode cells of a stem of a plant, a function of suppressing elongation of an internode of a neck of a plant, and/or a function of increasing inclination of a lamina of a plant.

[0047] Vectors used for the transformation of plant cells are not limited as long as the vector can express inserted genes in plant cells. For example, vectors comprising promoters for constitutive gene expression in plant cells (e.g., califlower mosaic virus 35S promoter) ; and promoters inducible by exogenous stimuli can be used. The term "plant cell" used herein includes various forms of plant cells, such as cultured cell suspensions, protoplasts, leaf sections, and callus.

[0048] A vector can be introduced into plant cells by known methods, such as the polyethylene glycol method, electroporation, *Agrobacterium* mediated transfer, and particle bombardment. Plants can be regenerated from transformed plant cells by known methods depending on the type of the plant cell (Toki et al., (1995) Plant Physiol. 100:1503-1507). For example, transformation and regeneration methods for rice plants include: (1) introducing genes into protoplasts using polyethylene glycol, and regenerating the plant body (suitable for *indica* rice cultivars) (Datta, S. K. (1995) in "Gene Transfer To Plants", Potrykus I and Spangenberg Eds., pp66-74); (2) introducing genes into protoplasts using electric pulse, and regenerating the plant body (suitable for *japonica* rice cultivars) (Toki et al (1992) Plant Physiol. 100, 1503-1507); (3) introducing genes directly into cells by the particle bombardment, and regenerating the plant body (Christou et al. (1991) Bio/Technology, 9: 957-962); (4) introducing genes using *Agrobacterium*, and regenerating the plant body; and so on. These methods are already established in the art and are widely used in the technical field of the present invention. Such methods can be suitably used for the present invention.

[0049] Once a transformed plant, wherein the DNA of the present invention is introduced into the genome, is obtained, it is possible to gain descendants from that plant body by sexual or vegetative propagation. Alternatively, plants can be mass-produced from breeding materials (for example, seeds, fruits, ears, tubers, tubercles, tubs, callus, protoplast, etc.) obtained from the plant, as well as descendants or clones thereof. Plant cells transformed with the DNA of the present invention, plant bodies including these cells, descendants and clones of the plant, as well as breeding materials obtained from the plant, its descendant and clones, are all included in the present invention.

[0050] The plant of the present invention is preferably a monocotyledonous plant, more preferably a plant of the Gramineae family, and most preferably a rice. The phenotype of the plant of the present invention is different from the

wild type phenotype. The phenotypes changed in the plants developed by the present invention include brassinosteroid sensitivity of a plant, plant growth such as internode cell elongation of the plant stem and internode elongation of the ear, inclination of leaves, and the positioning of microtubules perpendicular to the direction of internode cell elongation in the plant stem.

Brief Description of the Drawings

[0051]

Fig. 1 shows a schematic diagram of the internode elongation pattern of wild type rice and various dwarf mutant and wild-type rice plants. The relative lengths of the each internode to the stem are shown in the schematic diagram. Wild type is shown as N.

Fig. 2 represents photographs which show the phenotype of the *d61* mutants.

(A) Gross morphology. (Left) Wild type plant; (centre) *d61-1* mutant (weak allele); (right) *d61-2* mutant (strong allele).
(B) Elongation pattern of internodes. The wild type plant (left) shows the N-type of the elongation pattern, while the *d61-1* (centre) and *d61-2* (right) mutants show typical dn- and d6- type patterns, respectively.
(C) Panicle structure. The wild type plant (left) has a short panicle, while the *d61-1* (centre) and *d61-2* (right) mutants have longer panicles.
(D) Erect leaf of *d61*. The leaves of the wild type plant (left) are bent at the lamina joint indicated by the white arrow, while the leaves of *d61-1* (centre) and *d61-2* (right) mutants are more erect.
(E) Leaf sheath of *d61*. The leaf sheath in the *d61-1* (centre) and *d61-2* (right) mutants is shorter than in the wild type plants (left).

Fig. 3 represents microphotographs which show the structure of well-developed internodes from wild type and *d61-2* rice plants, as follows:

(A) longitudinal sections of the first internodes from wild type;
(B) longitudinal sections of the second internodes from wild type;
(C) longitudinal sections of the third internodes from wild type;
(D) longitudinal sections of the fourth internodes from wild type;
(E) longitudinal sections of the first internodes from *d61-2* rice plants;
(F) longitudinal sections of the second internodes from *d61-2* rice plants;
(G) longitudinal sections of the third internodes from *d61-2* rice plants; and
(H) longitudinal sections of the fourth internodes from *d61-2* rice plants.
Bar= 100 μm, respectively.

Fig. 4 are photographs and drawings which show the orientation of microtubules in elongating sells in the first internode of wild type and *d61-2* plants, consisting of immunofluorescence images (A and B) or schematic presentation (C and D) of the microtubule arrangement in internodal parenchyma cells of the first internode from wild type (A and C) and *d61-2* (B and D) plants. Bars= 50 μm.

Fig. 5 is a photograph which shows the response of seedlings of wild plant, *D61-1*, and *d61-2* to brassinolide.
Seeds were germinated on agar plates in the presence or absence of 1 μM brassinolide (BL). Seedlings were observed 1 day after germination. BL treatment induced abnormal growth in wild plant, while mutant seedlings were not affected thereto.

Fig. 6 is a photograph which shows effect of brassinolide on the degree of inclination of etiolated leaf lamina in wild type, *d61-1*, and *d61-2* plants.
The highest response of the leaf from wild type (panel A) and reduced response in mutant plants *d61-1* (panel B) and *d61-2* (panel C) are shown.

Fig. 7 is a drawing which shows amounts of brassinosteroids in wild type and *d61-2* rice plants, and biosynthetic precursors thereof.
The amounts (ng/g fresh weight) of each compound in mutant (upper) and wild type (lower) plants are shown. ND indicates not detected.

Fig. 8 is a photograph which shows de-etiolation phenotype of the *d61* in the dark.

(A) Wild type

Left: seedlings grown for two weeks in the dark
Right: seedlings grown for two weeks in the light

The internode elongation in wild type (A) and two gibberellin deficient rice mutants, *d18* (D) and *d35* (E), are indicated in the dark.
(B) *d61-1* mutant

Left: seedlings grown for two weeks in the dark
Right: seedlings grown for two weeks in the light

The white arrows indicate internode elongations in right of each panel, the dark condition. No elongation was observed in the light (left of each panel).
(C) *d61-2* mutant

Left: seedlings grown for two weeks in the dark
Right: seedlings grown for two weeks in the light
No internode elongation in *d61* mutant, *d61-1* (B) and *d61-2* (C), is observed even in the dark. The present inventors stripped the leaf sheath of plants grown in the dark.

(D) *d18* mutant

Left: seedlings grown for two weeks in the dark
Right: seedlings grown for two weeks in the light

(E) *d35* mutant

Left: seedlings grown for two weeks in the dark
Right: seedlings grown for two weeks in the light

Fig. 9 represents a drawing and photograph which show the strong linkage between the *d61* locus and *OsBRI1*.

(A) is a drawing which indicates map position of the *d61* locus on the long arm of chromosome 1.
(B) is a photograph which indicates the result of DNA hybridization analysis to test the linkage between the *d61* locus and *OsBRI1*. The RFLP of *OsBRI1* was observed between the *Japonica* parent T65 (lane T, 12.5 kb), and the *Indica* parent Kasarath (lane K, 17.5 kb) when the genomic DNAs were digested with *EcoR*I. Plants with a normal phenotype (WT) were heterozygous (12.5+ 17.5 kb) or homozygous for the *Indica* allele (17.5 kb) , while the plants with the mutant phenotype (mutant) were always homozygous for the *Japonica* allele (12.5 kb). Fig. 10 shows comparison of the deduced amino acid sequences of OsBRI1 and Arabidopsis BRI1. Identical residues are shaded. The underlined regions,. *1, *2, *3, and *4, indicate: a putative signal peptide, a leucine zipper motif, N, and C sides of a cysteine pair.

Fig. 11 is a continuation of Fig. 10.

Fig. 12 is a photograph which shows expression pattern of *OsBRI1* in various organs.
Total RNA (10 μg) from various organs of wild type plants were loaded into each lane.
Organ specific expression of *OsBRI1*

(A) Leaf blade (lane 1), leaf sheath (lane 2) , developed flower (lane 3), rachis (lane 4), shoot apex (lane 5), root (lane 6), and seed (lane 7).
Region specific expression of *OsBRI1* in the developing first internode

(B) Node (lane 1), divisional zone (lane 2), elongation zone (lane 3), and elongated zone (lane 4) in developing internodes.
Differential expression of *OsBRI1* in each elongating internode

(C) The divisional and elongation zones of the first to fourth internodes, respectively, at the actively elongating stage for each internode (lanes 1-4). *OsBRI1* was expressed at a high level also in the unelongated stem at the vegetative phase (lane 5).
Light-dependent and brassinolide-dependent expression of *OsBRI1*

(D) Rice seedlings were grown for ten days in the light (lanes 1 and 2) or dark (lane 3 and 4) on agar plate in the presence (lane 2 and 4) or absence (lane 1 and 3) of 1 μM brassinolide.

Fig. 13 is a photograph which shows phenotype of the transgenic rice plants expressing the antisense strand of *OsBRI1*.

(A) Dwarf phenotype of *OsBRI1* antisense plants with intermediate (centre) and severe phenotypes (right) compared to a wild type plant (left).
(B) Close up view of a transgenic plant with severe phenotype. Bar= 5 cm.
(C) Naked culm internodes of a transgenic plant. From left to right, wild type plant with normal elongation pattern of internodes and transgenic plants with the dm, dm-d6, and d6 phenotypes are shown, respectively.
(D) Leaf morphology of wild type (left) and transgenic plants with mild phenotype (right), showing the erect leaves in the latter.
(E) Abnormal leaf morphology of a transgenic plant with a severe phenotype, showing lack of developed sheath organs. Bar= 10 cm.
(F) Panicle morphology in wild type (left) and transgenic plants with the mild (centre) and intermediate (right) phenotypes

Fig. 14 is a photograph which shows the phenotype of a transgenic plant that expresses dominant negative of *OsBRI1*. The transgenic plant (left) and a control plant containing vector without any inserts (right) are shown.

Best Mode for Carrying out the Invention

[0052] The present invention is specifically illustrated below with reference to Examples, but it is not to be construed as being limited thereto.

[0053] Rice (*Oriza sativa*) seeds were soaked in distilled water and made to imbibe for 48 h at 30°C. After washing the seeds with distilled water several times, the seeds were germinated in a dark chamber at 30°C for 8 days. Rice plants were grown in the field or in the greenhouse at 30°C (day) and 24°C (night).

[Example 1] Characterization of rice *d61* dwarf mutants

[0054] Rice dwarf mutants *d61-1* and *d61-2* were obtained by treatment with N-methyl-N-nitrosourea (NMU), respectively (Fig. 2A).

[0055] *d61* mutants carrying the weak allele specifically fail to elongate the second internode (dm-type), while those with the strong allele fail to elongate all of the internodes except the uppermost one (d6-type) (Wu, X. et al. (1999) Bread. Sci. 49, 147-153).

[0056] The culm of *d61-2* is much shorter than that of *d61-1*. In addition, *d61-1* shows typical dm-type pattern of internode elongation, while *d61-2* shows the d6-type (Fig. 2B). Thus, they were initially characterized as two independent mutants. However, crossing test demonstrated that they are alleles on a single locus. This is the first example of rice mutants of a single locus which show different, specific patterns of inhibition of internode elongation.

[0057] These mutants have other abnormal phenotypes as a result of pleiotropic effect, as well as that inhibiting internode elongation specifically. For example, the neck internode of the mutants is longer than that of wild type plants (Fig. 2C). As the neck internode length shows an inverse relationship to the length of culm in these plants, wild type plant thus has the longest culm and shortest neck internode, the *d61-1* mutant has intermediate length culm and neck internodes, and *d61-2* has the shortest culm and the longest neck internode.

[0058] Another abnormal phenotype of these mutants is erect leaves (Fig. 2D) . In wild type plants, the leaf blade bends away from the vertical axis of the leaf sheath towards the abaxial side . The leaf blade bends away from the leaf sheath at a specific organ, lamina joint, which is indicated by arrows in Fig. 2D. When the leaf blades and sheaths are fully elongated, cells at the adaxial side of the lamina joint start to elongate causing the leaf blade to bend away form the leaf sheath. However, the leaf blade of a mutant does not clearly bend away from the leaf sheath. In the *d61-1* mutant some leaves still show slight bending (Fig. 2D, centre), but in the *d61-2* mutant almost all the leaves are completely erect (Fig. 2D, right).

[0059] That is, the degree of lamina inclination correlates to the severity of the dwarfism. The continuity of the severity

in lamina inclination suggests that the longitudinal elongation of the surface cells on the adaxial side of lamina, which causes the lamina inclination, responds continuously to the brassinosteroid signal.

[0060] The lack of bending of the mutant leaves is not caused by none or less development of the lamina joint. Indeed, even the *d61-2* mutant with the severe phenotype in lamina joint developed normally. The mutants showed shorter leaf sheaths than that of the wild type plants (Fig. 2E).

[Example 2] Observation of cell morphology in internode

[0061] Internode elongation is caused by cell division in the intercalary meristem and cell elongation in the elongation zone (Hoshikawa, K. (1989) Stem. In: The growing rice plant. (Nobunkyo), pp. 123-148). Therefore, dwarfing of the culms could be due to a defect in one or both of these processes. To distinguish between these possibilities, the present inventors examined sections of each internode from adult plants under the microscope.

[0062] Developing or developed culms at various stages were fixed in FAA (formalin: glacial acetic acid: 70% ethanol, 1:1:18), and dehydrated in a graded ethanol series. The samples were embedded in a Technovit 7100 resin (Kurzer, Germany) polymerized at 45°C and 3-5 μm sections were cut, stained with Toluidine Blue and observed under the light microscope.

[0063] Fig. 3 shows the cell morphology of the upper four internodes in a wild type plant and the *d61-2* mutant. In the wild type plant, cells in all internodes were longitudinally elongated and well organized with longitudinal files (Fig. 3A, 3B, 3C, and 3D). Similar longitudinal cell files were also seen in the first internodes of the mutant plants, although the cells were a little shorter than those in the wild type plant (Fig. 3E). In the non-elongated internodes of the mutant, such as the second and third internodes, the arrangement of cells was disorganized with no organized cell files apparent (Fig. 3F and 3G). Disorganization of the internodal cells indicates that the intercalary meristems of the mutants, which normally give rise to the longitudinal files of elongated cells, are not developed in the non-elongated internodes. In the fourth internode, organized cell files were present but the cells were much shorter than that of the wild type plant (compare Fig. 3D and 3H) . This suggests that intercalary meristems did develop in these internodes but the cells failed to elongate.

[Example 3] Observation of the arrangement of microtubules

[0064] It was described in detail that cell elongation depends on the orientation of microfibrils (Ledbetter, M. C. and Porter, K. R. (1963) J. Cell Biol. 19, 239-250; Green, P. B. (1962) Science. 138, 1404). Therefore, the present inventors examined the arrangement of microtubules in the internodal cells of wild type and *d61-2* mutant plants by immunofluorescence microscopy.

[0065] Microtubules in internodal parenchyma tissue were strained immunofluorescently. More specifically, internodal parenchyma tissue was prefixed for 45 min at room temperature in 3.7% (w/v) paraformaldehyde in microtubule-stabilizing buffer (0.1 M piperazine-diethanolsulphonic acid, 1 mM $MgCl_2$, 5 mM ethylenegiycol-bis(3-aminomethyl-ether-N,N,N',N'-tetraacetic acid, 0.2% (v/v) Triton X-100, 1% (w/v) glycerol, pH 6.9). Longitudinal sections were cut with a fresh razor blade, collected in the fixation solution, treated for 40 min therein, and washed in the fixation solution without paraformaldehyde. The sections were then incubated with a rabbit anti-α-tubulin monoclonal antibody, diluted 1:500 in phosphate-buffered saline containing 0.1% (v/v) Triton X-100, for 1 h at 37°C. The sections were washed three times in the buffer without anti-serum and then incubated overnight at 4°C with fluorescein-isothiocyanate-labeled mouse anti-rabbit IgG antibody diluted 1:50 in phosphate-buffered saline containing 0.1% (v/v) Triton X-100. After three washes in the same buffer, they were mounted in antifading solution (Fluoro Guard Antifade reagent, Bio-Rad) and observed under a fluorescence microscope.

[0066] As a result, in wild type plants, the microtubules in cells of elongating internodes were arranged in an orderly manner at right angles to the direction of elongation (Fig. 4A). However, in the *d61-2* mutant, the microtubules in cells of the first elongating internode were arranged in different directions in each cell, apparently at random (Fig. 4B). In addition, the microtubules in the mutant appeared to be thinner and less distinct relative to those of the wild type plant.

[0067] Moreover, the present inventors were unable to observe any organized microtubule arrangement in cells from non-elongated internodes of the mutant plants.

[0068] Taken together with the results in Example 2 show that non-elongated internodes in the mutants fail to develop an intercalary meristem and the cells lack an organized arrangement of microtubules. In those internodes that do elongate in the mutants, although less than in the wild type plants, an intercalary meristem does develop but the cells lack a well-organized microtubule arrangement.

[Example 4] Test of the sensitivity against brassinosteroids

[0069] The dwarf phenotype and erect leaves of the *d61* mutant suggests the possibility that the *D61 gene* product

could be involved in either the biosynthesis or signal transduction of brassinosteroids. Thus, the present inventors carried out the following experiments.

[0070] First, the present inventors attempted to restore the dwarfism of the *d61* mutants, but could not be achieved it by the application of brassinolide.

[0071] Next, seeds of the wild type and mutant plants were germinated on agar plates with or without 1 μM brassinolide. The characteristics of whole seedlings were observed 1 day after germination.

[0072] The result showed that, when the wild-type plants were germinated on the plates with brassinolide, the coleoptiles of wild type plants elongated abnormally resulting in a twisted shape and the foliage leaves grew poorly and did not break through the coleoptile (Fig. 5). Furthermore, root elongation was inhibited and thus the roots were not straight but developed in a wavy form. The wild type plants grew normally in the absence of brassinolide, with coleoptile elongation stopping at an early stage of germination and then foliage leaves elongating to break out of the coleoptile. Roots developed normally and did not have any wavy form (Fig. 5). In contrast to the wild type plants, the mutants showed normal growth patterns, as well as that of the wild type plants on plates without brassinosteroids, even in the presence of brassinosteroids. These results suggest that the mutant plants are less sensitive to brassinolide than the wild type plants.

[0073] The present inventors further tested the sensitivity of the mutants to brassinolide using a more quantitative method.

[0074] The degree of bending between the leaf blade and leaf sheath in rice is well known to be sensitive to the concentration of brassinolide or its related compounds. This unusual character of rice leaf is the basis for a quantitative bioassay for brassinosteroids, known as the lamina joint test (Wada, K et al. (1981) Plant and Cell Physiol. 22, 323-325), even though the biological function of endogenous brassinosteroids in monocotyledonous plants including rice remains unknown. If the mutants are less sensitive to brassinosteroids, the degree of bending between the leaf blade and sheath of the mutant plants will be less than that of the wild type plants.

[0075] Lamina joints of first leave of wild type T65 plant (the background strain of the mutants), *d61-1* mutant, and *d61-2* mutant were tested with 10^{-3} and 10^{-2} μg/ml of brassinosteroids, respectively.

[0076] As a result, the leaf blade of wild type T65 plants was bent almost at right angles to the axis of the leaf sheath in the absence of exogenous brassinolide becoming even more bent in the presence of increasing concentrations of brassinolide (Fig. 6A). When the mutants were used for the test, the degree of bending increased with higher concentrations of brassinolide as in the wild type plants. However, the absolute degree of bending of the leaves from the mutants was much less than that of the wild type plants under the same conditions (Fig. 6B and C). This was particularly evident with the *d61-2* mutant in which control leaves were almost straight and leaves treated with 10^{-2} μg/ml brassinolide were bent at less than a right angle to the leaf sheath axis. The results of the lamina joint test confirm that the sensitivity of the mutants to brassinosteroids is less than that of the wild type plants.

[Example 5] Quantitative analysis of brassinosteroids in the *d61-2* mutant

[0077] Example 4 demonstrates that the mutants have reduced sensitivity to brassinosteroids. That is, mutants may synthesize higher concentrations of brassinosteroids to compensate for the reduction. Thus, the present inventors measured the concentration of brassinosteroids in both the *d61-2* mutant and wild type plants using GC-SIM with internal standards.

[0078] Wild type and *d61-2* plants were grown in a greenhouse with a 16-hrs day and 8-hrs night. Shoots from 2-month old plants were harvested and then immediately lyophilized. Lyophilized shoots (50 g fresh weight equivalent) were extracted twice with 250 ml of MeOH-$CHCl_3$ (4:1 [v/v]), and deuterium-labeled internal standards (1 ng/g fresh weight) were added thereto. The extract was partitioned between $CHCl_3$ and H_2O after evaporation of the solvent *in vacuo*. The $CHCl_3$-soluble fraction was subjected to silica gel chromatography (Wako-gel C-300; Wako; 15 g) . The column was sequentially eluted with 150 ml each of $CHCl_3$ containing 2% methanol and $CHCl_3$ containing 7% methanol. Each fraction was purified by Sephadex LH-20 column chromatography, where the column volume was 200 ml and the column was eluted with methanol-$CHCl_3$ (4:1 [v/v]). The fractions eluting from 0.6 to 0.8 (V_e/V_t) were collected as brassinosteroid fractions. After pre-purification on an CDS cartridge column (10x 50 mm [internal diameter x column length]) in MeOH, the eluates derived from 7% MeOH fractions were subjected to ODS-HPLC at a flow rate of 8 ml/min with 65% acetonitrile as the solvent. In HPLC purification, the 7% methanol eluate was resolved into castasterone (retention time from 10 to 15 min), typhasterol (25 to 30 min), 6-deoxocastasterone (40 to 45 min) fractions, and the 2% methanol eluate gave a 6-deoxotyphasterol fraction (55 to 60 min). Each fraction was derivatized and analyzed by GC-SIM. The endogenous levels of brassinosteroids were calculated from the ratio of the peak areas of the prominent ions from the endogenous brassinosteroids and the internal standard.

[0079] As a result, brassinosteroid was not detected in shoots from either the mutant or wild type plants, suggesting that brassinolide is a minor component of the total brassinosteroids pool. However, all of the other brassinosteroids were detected in both plant types, with the exception of teasterone which was not found in the wild type plants. The

contents of all of the brassinosteroid compounds detected were greater in the mutant plants (Fig. 7). In particular, castasterone was four times higher in the mutant than in the wild plant. These results support the hypothesis that the mutants have no sensitivity to brassinosteroids.

[Example 6] de-etiolation phenotype of the *d61* mutants

[0080] Reduction of hypocotyl elongation and emergence of opening of the cotyledons and primary leaves in complete darkness are reported in *Arabidopsis* mutants with deficiencies in brassinosteroid biosynthesis or brassinosteroid signaling when grown in the dark (Kauschmann, A et al. (1996) Plant J. 9, 701-703; Szekeres, M. et al. (1996) Cell. 85, 171-182).

[0081] This de-etiolated (DET) or constitutive photomorphogenesis (COP) phenotype in darkness is a common feature of Arabidopsis brassinosteroids-related mutants. A similar DET or COP phenotype is also observed in a tomato dwarf (d) mutant that shows a short hypocotyl, lack of apical hook, and expansion of cotyledons (Bishop, G. J. et al. (1996) Plant Cell. 8, 959-969). In contrast, a pea brassinosteroids-defective mutant, *lkb,* does not show such a DET phenotype (Nomura, T. et al. (1997) Plant Physiol. 93, 572-577). Mutants were grown in the dark to determine whether such DET or COP phenotypes were also found in monocotyledonous plants and whether the *d61* rice mutants showed characteristics of skotomorphogenesis.

[0082] As a result, when wild type plants were germinated in the dark, they showed unusual elongation of the mesocotyl and internodes compared to light-grown seedlings (Fig. 8A). Such elongation of the mesocotyl and internodes did not occur in the mutants even in the dark (Fig. 8B and 8C). This failure of the mesocotyl and internodes to elongate in the dark is not a common characteristic of rice dwarf mutants. For example, two other dwarf mutants, *d18* and *d35,* which are deficient in gibberellin biosynthesis, showed elongated mesocotyls and internodes when grown in the dark (Fig. 8D and 8E, respectively).

[0083] Thus, it is conceivable that the reduced elongation of the mesocotyl and internodes are specific feature of the *d61* mutants and that the *d61* mutants have de-etiolated phenotype. In addition, it is also indicated that de-etiolation due to defects in brassinosteroid signal is common characteristic in both dicotyledonous and monocotyledonous plants. That is, it is conceivable that brassinosteroid signals are important for skotomorphogenesis in both dicotyledonous and monocotyledonous plants.

[Example 7] Mapping and linkage analysis of *D61* locus

[0084] For mapping of the *D61* locus, the present inventors crossed the *d61-2* mutant with an *Indica* rice cultivar, Kasarath (*Oriza sativa* L. cv. *Kasarath*). The linkage analysis between the mutant phenotype and restriction fragment polymorphism (RFLP) markers released from the Rice Genome Project (Tsukuba, Japan) revealed that the *D61* locus maps to the long arm of chromosome 1, with tight linkage to the RFLP marker, C1370 (Fig. 9A).

[0085] As *d61* could be characterized as a mutant with no or reduced sensitivity to brassinosteroid, the present inventors also tested the linkage between the mutant phenotype and a rice gene that is homologous to the Arabidopsis *BRI1* gene.

[0086] The Arabidopsis *BRI1* gene was isolated as the only gene that is involved in brassinosteroid signal transduction (Li, J. and Chory, J. (1997) Cell. 90, 929-938). Furthermore, the present inventors carried out a BLAST search to identify one rice EST clone, S1676, with high homology to the Arabidopsis *BRI1* gene (Li, J. and Chory, J. (1997) Cell. 90, 929-938).

[0087] Rice genomic DNA was isolated from leaf tissue using an ISOPLANT DNA isolation kit (Nippon GENE Co., Japan). One μg of the genomic DNA was digested with appropriate restriction enzymes and transferred onto Hybond N^+ membranes (Amersham) under alkaline conditions. The membrane was probed using the partial cDNA fragment (corresponding to the region from Ser 740 to Asp 1116 in the kinase domain), which specifically hybridized with the genomic DNA fragment encoding *OsBRI1*. All of the steps were carried out according to the method described by Church and Gilbert (1984) PNAS. 81, 1991-1995, except that membranes were hybridized at high stringency (68°C).

[0088] An RFLP between the *Japonica* (12.5 kb) and *Indica* (17.5 kb) rice was observed when genomic DNAs were digested with EcoRI and probed with the cDNA clone. All F2 plants with the mutant phenotype were homozygous for the *Japonica* allele (12.5 kb), whereas the F2 plants with the wild type phenotype were either homozygous for the *Indica* allele (17.5 kb) or heterozygous with both the *Japonica* and *Indica* alleles (12.5+ 17.5 kb, Fig. 9B). This result demonstrates that the *D61* locus is closely linked to the position of the rice gene that is homologous to the Arabidopsis *BRI1*.

[Example 8] Identification of the *d61* gene

[0089] The above linkage analysis strongly suggested that the *d61* mutation is caused by loss of function of the rice

homologue of the Arabidopsis *BRI1* gene. To test this possibility, the present inventors screened rice genomic DNA library with probes and isolated the entire length. of the rice *BRI1* homologous gene (*OsBRI1*, *Oryza sativa BRI1*). Hybridization in this screening was performed as described in Church and Gilbert (1984) except that membranes were hybridized at higher stringency (68°C). Sequencing was carried out according to the same method described by Church and Gilbert.

[0090] The structure of the *OsBRI1* gene is quite similar to the Arabidopsis *BRI1* gene in its entire length (Fig. 10 and Fig. 11). The predicted OsBRI1 polypeptide contains several domains that are also present in the BRI1, and the functions of which were discussed (Li, J. and Chory, J. (1997) Cell. 90, 929-938). These domains consist of a putative signal peptide, two conservatively spaced cysteine pairs, a leucine-rich repeat domain, a transmembrane domain, and a kinase domain. The N-terminus of the predicted OsBRI1 polypeptide has a hydrophobic segment which is predicted to act as a signal peptide to transport the protein to the plasma membrane. In the BRI1 protein, Li and Chory predicted a potential 4-hepted amphipathic leucine zipper motif following the signal peptide (Li, J. and Chory, J. (1997) Cell. 90, 929-938), but OsBRI1 does not have such a typical leucine zipper motif in the corresponding region.

[0091] A putative extracellular domain (from Met^{1} to Leu^{670}), consisting of 22 tandem copies of a leucine-rich repeat (LRR) of about 24-amino acids with 12 potential N-glycosylation sites (Asn-X-Ser/Thr), is flanked by pairs of conservatively spaced cysteines. The LRR has been implicated to function in protein-protein interactions (Kobe, B. and Deisenhofer, J. (1994) Trends Biochem. Science. 19, 415-421).

[0092] In comparison to the BRI1 sequence, OsBRI1 lacks three LRR domains corresponding to the third to fifth repeat of the Arabidopsis BRI1. The two LRRs before this deletion are less conserved except for the consensus residues found between other LRR proteins, but the LRRs of both proteins are well conserved after the deletion in both the LRR consensus residues and non-conservative amino acids. An unusual feature of the LRR region of BRI1 is the presence of a 70-amino acid island between the 21st and 22nd LRR (Li, J. and Chory, J. (1997) Cell. 90, 929-938). A highly similar feature is also present in OsBRI1 with the same number of amino acids between the 18th and 19th LRRs corresponding to the site of island in BRI1. This unusual amino acid island in LRR region must be important for functions thereof, because exchange of an amino acid residue in this island resulted in the loss of function of *BRI1* (Li, J. and Chory, J. (1997) Cell. 90, 929-938). This motif was thought to be important for direct interaction with brassinosterolds or for maintaining the structure of the brassinosteroids-binding domain (Li, J. and Chory, J. (1997) Cell. 90, 929-938).

[0093] The protein kinase domain of OsBRI1 has all eleven conserved subdomains of eukaryotic protein kinases, retaining the invariant amino acid residues in their proper positions (Hanks, S. K. and Quinn, A. M. (1991) Meth.Enzymol. 200, 38-62). The protein kinase domain of OsBRI1 is highly related to that of BRI1 (44%) over the entire region. It is also related to the kinase domains of other receptor-like protein kinases in higher plants such as ERECTA (Torii, K. U. et al. (1996) Plant Cell. 8, 735-746), CLV1 (Clark, S. E. et al. (1997) Cell. 3, 575-585), and RLK5 (Walker, J. C. (1993) Plant J. 3, 451-456) from Arabidopsis, and Xa21 from rice (Song et al., 1995). The highly conserved structure of OsBRI1 and these receptor-like protein kinases, especially in subdomains VIb and VIII, suggests that OsBRI1 is a serine/threonine kinase rather than a tyrosine kinase (Hanks, S. K. and Quinn, A. M. (1991) Meth. Enzymol. 200, 38-62).

[Example 9] Sequencing of *OsBRI1* gene in *d61-1* and *d61-2* mutants

[0094] The present inventors also determined the entire sequences of the *OsBRI1* gene in the *d61-1* and *d61-2* mutants, and compared them to that of the wild type plant. The present inventors identified a single nucleotide substitution in each mutant allele at different sites (Table 1). The genomic mutation in *d61-1* resulted in exchange from threonine to isoleucine at residue 989 in subdomain IX of the kinase domain which is conserved between OsBRI1 and BRI1. The genomic mutation in *d61-2* changed valine to methionine at residue 491 in the 17th LRR, just before the unusual 70-amino acid interrupting region. These mutations in the *OsBRI1* genes from the *d61* mutants provide strong evidence that *OsBRI1* encodes the *D61* locus.

Table 1

Alleles	Characteristics of mutation	Position of coding sequence
d61-1	C → T	Thr → Ile (989)
d61-2	G → A	Val → Met (491)

[Example 10] Molecular complementation analysis of the *d61* mutation by the introduction of *OsBRI1* gene

[0095] To confirm that *OsBRI1* corresponds to the *d61* locus, the present inventors carried out complementation

analysis of the *d61-1* mutant by introduction of the wild-type *OsBRI1* gene.

[0096] More specifically, to confirm complementarity of *d61* phenotype due to introduction of a genomic *OsBRI1* clone including its 5' and 3' flanking regions, a 10.5-kb restriction fragment including the entire coding region was cloned into the *Xba*I-*Sma*I sites of the hygromycin resistance binary vector pBI101-Hm3 (Sato, Y. et al. (1999) EMBO J. 18, 992-1002). pBI-cont was used as a control vector. The present inventors performed rice tissue culture and *Agrobacterium tumefaciens* mediated transformation.

[0097] Transformation of *d61* with a control vector that carries no rice genomic DNA had no apparent effect on the culm length or the structure of leaves. However, when a 10.5 kb DNA fragment containing the entire wild-type *OsBRI1* gene was introduced, the normal phenotype was recovered in almost plants that were resistant to hygromycin. This result confirms that the *d61* mutant phenotype is caused by the loss-of-function mutation in the *OsBRI1* gene.

[Example 11] RNA hybridization analysis of *OsBRI1*

[0098] Nothing is known about the function of endogenous brassinosteroids in monocotyledonous plants. Therefore, the present inventors tested the expression pattern of the *OsBRI1* gene in various rice organs by RNA hybridization analysis.

[0099] RNA was isolated from various rice tissues as described in literature (Chomczynski, P. and Sacchi, N.: Anal. Biochem. (1987) 162:156). Ten μg of total RNA were electrophoresed in a 1% agarose gel, then transferred to a Hybond N^+ membrane (Amersham) , and analyzed by RNA gel blot hybridization. The present inventors used a partial cDNA fragment (Ser^{740} to Asp^{1116} corresponding to the kinase domain), as a probe which specifically hybridized to the genomic DNA fragment encoding *OsBRI1*. DNA hybridization analysis was performed with the same probe and hybridization conditions as described above.

[0100] As a result, a single, strongly-hybridizing band was detected in RNA from vegetative shoot apices (Fig. 12A). The size of the band was approximately 3.5 kb, which is almost the same size as the longest cDNA clone. More weakly-hybridizing bands of the same size were also observed in RNA from flowers, rachis, roots, and expanded leaf sheaths, while no or very faint bands were observed in RNA from expanded leaf blades. Thus, the expression of *OsBRI1* varies markedly between organs suggesting that the sensitivity to brassinosteroids also differs among these organs.

[0101] The present inventors also examined the expression of *OsBRI1* in elongating culms (Fig. 12B). Elongating culms were divided into four parts: the node and the division, elongation, and elongated zones of the internode. As a result, the most strongly hybridizing band was found in RNA from the division zone. RNA from the elongation zone also gave a strong signal. RNA from the node gave only a weak signal, whilst that from the elongated internode gave no signal at all. These results indicate that the elongating culm has different sensitivities to brassinosteroids partially, with the most sensitive parts being the division and elongation zones where cells are actively dividing and elongating. The present inventors further examined the expression of *OsBRI1* in the elongation zones of the upper four internodes at the stage when each internode was actively elongating. A strongly hybridizing band was found in RNA from the elongation zone of the uppermost (first) and the lowest (fourth) internodes, while relatively weak bands were seen with the second and third internodes (Fig. 12C). This result indicates that the internodes differ in their sensitivity to brassinosteroids, with the second and third internodes being the least sensitive.

[0102] It was observed that the internodes differ in their sensitivity to brassinosteroids. It suggests that the uppermost and fourth internodes have higher sensitivity to brassinosteroids than the second and third internodes, if the amount of *OsBRI1* is a limiting factor in brassinosteroid signal transduction. This idea is supported by the mutant allele with the intermediate, dm-d6 type phenotype. These plants show specific reduction of the second and third internodes while the uppermost and fourth internodes are elongated. This is consistent with the second and third internodes, with lower expression level of *OsBRI1*, having lower sensitivity to brassinosteroids such that they are unable to respond to the brassinosteroid signal and elongate. Presumably, the higher *OsBRI1* expression level in the uppermost and fourth internodes does allow these internodes to respond to the brassinosteroid signal and elongate. The higher expression level of *OsBRI1* in the uppermost and fourth internodes can explain the unusual internode elongation pattern of the dm-d6 type, but it cannot explain the occurrence of the d6 or dm type. The d6 type, in which all of the internodes except the uppermost are reduced, could indicate that the uppermost internode is exposed to higher levels of brassinosteroids than the fourth internode. The timing of the elongation of the uppermost internode corresponds with the development of anthers in the flowers, and high level of brassinosteroids have been observed in these organs in many plants (Grove, M. et al. (1979) Nature, 281, 216-217; Plattner, D. et al. (1986) J. Natural Products. 49, 540-545; Ikekawa, N. et al. (1988) Chem. Pharm. Bull. 36, 405-407; Takatuto, S. et al. (1989b) Agric. Biol. Chem. 53, 2177-2180; Suzuki, Y. et al. (1986) Agric. Biol. Chem. 50, 3133-3138; Gamoh, K. et al. (1990) Anal. Chim. Acta. 228, 101-105). It appears that high levels of brassinosteroids move down from the anthers to lower organs , such as , the uppermost internode and induce internode elongation and that the fourth internode completes its elongation before flower development and does not receive the high level brassinosteroid signal from the flowers at the time of its active elongation. The brassinosteroid level and the sensitivity to brassinosteroids of *OsBRI1* cannot explain the specific retardation of the second internode

observed in the dm-type mutants. Therefore, some other factor(s) must be involved. It seems likely that elongation of the second internode is regulated by several factors, since there are several independent dwarf mutants with the dm-phenotype including *d1, d2, d11*, and *d61*.

[0103] Very recently, the *D1* gene was isolated and found to encode a protein with a similar structure to the α subunit (G-α) of a G protein (Fujisawa, Y. et al. (1999) Proc. Natl. Acad. Sci. USA. 96, 7575-7580; Ashikari, M. et al. (1999) Proc. Natl. Acad. Sci. USA. 96, 10284-10289). The D1 G-α like protein is now thought to be involved in the gibberellin (GA) signal transduction pathway, since the *d1* mutant alleles show low or no sensitivity to active GA. It is interesting that the loss-of-function mutants of the brassinosteroid signal-related protein, OsBRI1, and that of the GA signal-related protein, Gα, show the same phenotype, i.e., specific retardation of the second internode. Thus, in the induction of elongation in the second internode, there could be a specific mechanism common to brassinosteroids and GA signal transduction in the second internode.

[0104] Interestingly, high level expression of *OsBRI1* was also seen in the stem at the vegetative stage, in which the internodes do not elongate, showing that high level expression of *OsBRI1* in the culm does not necessarily coincide with internode elongation.

[Example 12] Effect of exogenous brassinolide and light on the level of *OsBRI1* mRNA

[0105] The present inventors also tested the effects of exogenously applied brassinolide and light on the level of *OsBRI1* mRNA. Germinating seeds were placed on 0.9% agar plates with or without 1 μM brassinolide and grown for six days in the light or dark.

[0106] As a result, on plates without brassinosteroids, the expression level of *OsBRI1* in dark grown seedlings was higher than in light grown seedlings (Fig. 12D).

[0107] This suggests that the dark-grown rice seedlings have a higher sensitivity to brassinosteroids than the light-grown plants (Worley, J. F. and Mitchell, J. W. (1971) J. Amer. Soc. Hort. Sci. 96, 270-273). High sensitivity to brassinosteroids in dark-grown plants will due to the elongation of internode cells in situations where the cells in light-grown plants do not respond to brassinosteroids.

[0108] Furthermore, on plates with brassinosteroids, both of the light-and the dark-grown rice seedlings had a reduced level of *OsBRI1* expression.

[0109] In contrast to rice, the level of *BRI1* expression in Arabidopsis is little changed between dark- and light-grown seedlings (Li, J. and Chory, J. (1997) Cell. 90, 929-938). The reason for this difference between the expression patterns of the rice *OsBRI1* and that of the Arabidopsis *BRI1* is not known. However, the difference could be related to the difference in photoresponse mechanisms that rice is short-day plant, while Arabidopsis is long-day plant.

[Example 13] Phenotypic analysis of transgenic plants expressing the antisense strand of *OsBRI1*

[0110] The above phenotypic analyses of the *d61* mutants and the single nucleotide exchange in the *OsBRI1* genes in each mutant suggest that they might not be null alleles and could retain some partial function. To investigate further the function of brassinosteroids in rice, the present inventors attempted to generate other mutants with more severe phenotypes by overexpression of the antisense strand of the *OsBRI1* transcript under the control of the rice *Actin1* gene promoter (Zang, W. et al. (1991) Plant Cell. 3, 1155-1165).

[0111] For constructing *Actin1* promoter::antisense *OsBRI1*, a promoter-terminator cassette (pBIActInos) containing the *Act1* promoter (Zhang, W. et al. (1991) Plant Cell. 3, 1155-1165) and *NOS* terminator was constructed by substitution of the *Act1* promoter for the 35S promoter in the hygromycin resistance binary vector, pBI35Snos (Sato, Y. et al. (1999) EMBO J. 18, 992-1002), which contains the 35S promoter and NOS terminator, between the *Hind*III and *Xba*I sites. The cDNA clone encoding entire *OsBRI1* coding region was introduced between the *Xba*I and *Sma*I sites of pBIActInos. Vector pBI-cont, containing no insert was used as a control vector.

For reduction of *OsBRI1* expression, the *OsBRI1*-antisense cDNA were introduced into the rice cultivar *Nipponbare.* The present inventors performed rice tissue culture and *Agrobacterium tumefaciens* mediated transformation.

[0112] Almost all of the resulting transgenic plants (90% or more, 18 out of 20) produced erect leaves during the early stages of seedling growth (Fig. 13D). All of the transformants (20 out of 20) showed a dwarfed phenotype of varying severity (Fig. 13A). In the plants with the weakest phenotype, the length of each internode was partially and uniformly reduced resulting in an elongation pattern similar to that of the wild plant (Fig. 13C) (dn-type mutants). Plants with intermediate phenotypes had the typical internode elongation patterns of dm-type (specific reduction of the second internode, Fig. 13C) or d6-type (specific reduction of the second to fourth internodes, Fig. 13C) mutants or a mixed dm- and d6-type phenotype (specific reduction of the second and third internodes, Fig. 13C). Plants with the severe phenotype only formed abnormal leaves without developed sheath organs and the internodes did not elongate (Fig. 13E). These kinds of plants were less than 15-cm high, even after cultivated for one year or more, and did not produce seeds (Fig. 13B). The other phenotypes were inherited in subsequent generations and cosegregated with hygromycin

resistance. The cosegregation between the abnormal phenotypes and hygromycin resistance, and the similarity between the intermediate phenotype of the antisense plants and the *d61* mutants demonstrate that the antisense strand acts to suppress the function of *OsBRI1* in the transgenic plants.

[Example 14] Phenotype of transgenic plants expressing dominant negative of *OsBRI1*

[0113] Transgenic rice containing the kinase region of the *OsBRI1* gene under control of the rice actin 1 gene promoter (Zhang, W. et al., (1991) Plant Cell, 3: 1155-1165) was produced to analyze the function of the rice brassinosteroid receptor. That is, the plasmid was constructed as follows so that only the carboxy terminal kinase domain of brassinosteroid receptor would be expressed without the amino terminus region from the first methionine to 738th glycine. The present inventors used a pair of the primer, 5'-GGCTCTAGACAGCCATGGCGAGCAAGCGGCGGAGGCTG-3' /SEQ ID NO: 4 (5'-primer: which includes TCTAGA as XhoI site, CAGCC added to increase translation efficiency, ATG as the initiation codon, an additional GCG encoding alanine, and AGC encoding 739th serine, following further nucleotides encoding amino acids after 740th residue of the wild type sequence, lysine, arginine, arginine, and leucine) and 5'-AGATCTACTCCTATAGGTA-3' /SEQ ID NO: 5 (3'-primer: which includes AGATCT as XbaI site and following 3'-untranslated region). These two primers were used to amplify the kinase region of the brassinosteroid receptor. The amplified fragment was then digested with XbaI and inserted into a pBI vector between XbaI-SmaI sites. The vector pBI-cont which does not contain the insert was used as a control.

[0114] The rice cultivar Nipponbare was used to produce transgenic plants which expresses kinase domain to control *OsBRI1* expression. Rice tissue culture and *Agrobacterium tumefaciens* mediated transformation were performed.

[0115] As a result, most of the transgenic plants (90% or more: 25/27 individuals) formed erect leaves at an early stage of seeding growth (Fig. 14). The length between the internodes was partially and equally shortened. The phenotype was inherited to the progeny by co-segregation with hygromycin resistance activity. The co-segregation between the abnormal phenotype and hygromycin resistance and similarities between the dominant negative plants and the *d61* mutant intermediate phenotype indicate that the partial cDNA for the kinase portion has activity in the transgenic plant and suppresses OsBRI1 function.

Industrial Applicability

[0116] The present invention provides a gene and a protein which functions to increase rice brassinosteroid sensitivity. This gene is involved in elongation of plant internode cells and inclination of leaves. Therefore, it is possible to produce phenotypically modified plants by controlling this gene. For example, by suppressing the expression of the gene of the present invention, dwarf plants, which are resistant to lodging and which enables planting a higher number of individuals per unit area, can be produced, which is significant in the production of crop products. It is also possible to produce ornamental plants having new aesthetic value by dwarfism of height or culm length of said plants via suppression of expression of DNA of the present invention. On the other hand, brassinosteroid sensitivity of the plant can be increased by introducing and expressing the DNA of the present invention in plants, resulting in increase of height of the plant and yield of the whole plant. This is useful especially in increasing yield of plants for animal feed.

SEQUENCE LISTING

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Ser Asn Arg Leu Ser Gly Pro Ile Pro Ser Trp Leu Gly Lys Leu Ser
450 455 460

tac ttg gct atc ttg aag ctc agc aac aat tcc ttc tcg ggg cct ata 1440
Tyr Leu Ala Ile Leu Lys Leu Ser Asn Asn Ser Phe Ser Gly Pro Ile
465 470 475 480

ccg cca gag ctc ggt gac tgc cag agc ttg gtg tgg ctg gac ctg aat 1488
Pro Pro Glu Leu Gly Asp Cys Gln Ser Leu Val Trp Leu Asp Leu Asn
485 490 495

agc aat cag ctg aat gga tca ata ccc aaa gag ctg gcc aaa cag tct 1536
Ser Asn Gln Leu Asn Gly Ser Ile Pro Lys Glu Leu Ala Lys Gln Ser
500 505 510

ggg aag atg aat gtt ggc ctc ata gtt gga cgg cct tac gtt tat ctt 1584
Gly Lys Met Asn Val Gly Leu Ile Val Gly Arg Pro Tyr Val Tyr Leu
515 520 525

cgc aac gac gag ctg agc agc gag tgc cgt ggc aag ggg agc ttg ctg 1632
Arg Asn Asp Glu Leu Ser Ser Glu Cys Arg Gly Lys Gly Ser Leu Leu
530 535 540

gag ttt acc agc atc cga cct gat gac ctc agt cgg atg ccg agc aag 1680
Glu Phe Thr Ser Ile Arg Pro Asp Asp Leu Ser Arg Met Pro Ser Lys
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Lys Leu Cys Asn Phe Thr Arg Met Tyr Val Gly Ser Thr Glu Tyr Thr
565 570 575

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Phe Asn Lys Asn Gly Ser Met Ile Phe Leu Asp Leu Ser Tyr Asn Gln
580 585 590

ctg gac tcg gcg att cct ggc gag ctg ggg gac atg ttc tac ctc atg 1824
Leu Asp Ser Ala Ile Pro Gly Glu Leu Gly Asp Met Phe Tyr Leu Met
595 600 605

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Ile Met Asn Leu Gly His Asn Leu Leu Ser Gly Thr Ile Pro Ser Arg

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625 630 635 640

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645 650 655

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660 665 670

tcc ctt gcc aca ttt ccg aag agc cag tat gag aat aac act ggt tta 2064
Ser Leu Ala Thr Phe Pro Lys Ser Gln Tyr Glu Asn Asn Thr Gly Leu
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tgt ggc ttc cca ctg cca cca tgt gac cat agt tcc cca aga tct tcc 2112
Cys Gly Phe Pro Leu Pro Pro Cys Asp His Ser Ser Pro Arg Ser Ser
690 695 700

aat gac cac caa tcc cac cgg agg cag gca tcg atg gca agc agt atc 2160
Asn Asp His Gln Ser His Arg Arg Gln Ala Ser Met Ala Ser Ser Ile
705 710 715 720

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Ala Met Gly Leu Leu Phe Ser Leu Phe Cys Ile Ile Val Ile Ile Ile
725 730 735

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Ser Arg Asp Ile Tyr Ile Asp Ser Arg Ser His Ser Ala Thr Met Asn
755 760 765

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gtt gag gcc aca aat ggc ttc cac atc gca tgc caa att ggg tct ggt 2448
Val Glu Ala Thr Asn Gly Phe His Ile Ala Cys Gln Ile Gly Ser Gly
805 810 815

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Gly Phe Gly Asp Val Tyr Lys Ala Gln Leu Lys Asp Gly Lys Val Val

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Ala Ile Lys Lys Leu Ile His Val Ser Gly Gln Gly Asp Arg Glu Phe
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act gca gaa atg gag acc att ggc aag atc aaa cac cgt aac ctt gtt 2592
Thr Ala Glu Met Glu Thr Ile Gly Lys Ile Lys His Arg Asn Leu Val
850 855 860

cca ctt ctt ggc tat tgc aag gct ggt gag gag cgg ttg ttg gtg tat 2640
Pro Leu Leu Gly Tyr Cys Lys Ala Gly Glu Glu Arg Leu Leu Val Tyr
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Asp Tyr Met Lys Phe Gly Ser Leu Glu Asp Val Leu His Asp Arg Lys
885 890 895

aag atc ggt aaa aag ctg aat tgg gag gca aga cgg aaa atc gct gtt 2736
Lys Ile Gly Lys Lys Leu Asn Trp Glu Ala Arg Arg Lys Ile Ala Val
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Gly Ala Ala Arg Gly Leu Ala Phe Leu His His Asn Cys Ile Pro His
915 920 925

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Ile Ile His Arg Asp Met Lys Ser Ser Asn Val Leu Ile Asp Glu Gln
930 935 940

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Val Pro Pro Glu Tyr Tyr Gln Ser Phe Arg Cys Thr Thr Lys Gly Asp
980 985 990

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Val Tyr Ser Tyr Gly Val Val Leu Leu Glu Leu Leu Thr Gly Lys Pro
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cta ctc aag gag gat cca tcc gtc gag ctt gag ctg ctg gag cat ttg 3168
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Lys Ile Ala Cys Ala Cys Leu Asp Asp Arg Pro Ser Arg Arg Pro Thr
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Gly Gly Asp Gly Ala Cys Arg Phe Pro Gly Ala Gly Cys Arg Asn Gly

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Arg Leu Thr Ser Leu Ser Leu Ala Gly Val Pro Leu Asn Ala Glu Phe
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Arg Ala Val Ala Ala Thr Leu Leu Gln Leu Gly Ser Val Glu Val Leu
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Ser Leu Arg Gly Ala Asn Val Ser Gly Ala Leu Ser Ala Ala Gly Gly
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Ala Arg Cys Gly Ser Lys Leu Gln Ala Leu Asp Leu Ser Gly Asn Ala
115 120 125

Ala Leu Arg Gly Ser Val Ala Asp Val Ala Ala Leu Ala Ser Ala Cys
130 135 140

Gly Gly Leu Lys Thr Leu Asn Leu Ser Gly Asp Ala Val Gly Ala Ala
145 150 155 160

Lys Val Gly Gly Gly Gly Gly Pro Gly Phe Ala Gly Leu Asp Ser Leu
165 170 175

Asp Leu Ser Asn Asn Lys Ile Thr Asp Asp Ser Asp Leu Arg Trp Met
180 185 190

Val Asp Ala Gly Val Gly Ala Val Arg Trp Leu Asp Leu Ala Leu Asn
195 200 205

Arg Ile Ser Gly Val Pro Glu Phe Thr Asn Cys Ser Gly Leu Gln Tyr
210 215 220

Leu Asp Leu Ser Gly Asn Leu Ile Val Gly Glu Val Pro Gly Gly Ala
225 230 235 240

Leu Ser Asp Cys Arg Gly Leu Lys Val Leu Asn Leu Ser Phe Asn His
245 250 255

Leu Ala Gly Val Phe Pro Pro Asp Ile Ala Gly Leu Thr Ser Leu Asn
260 265 270

Ala Leu Asn Leu Ser Asn Asn Asn Phe Ser Gly Glu Leu Pro Gly Glu
275 280 285

Ala Phe Ala Lys Leu Gln Gln Leu Thr Ala Leu Ser Leu Ser Phe Asn
290 295 300

His Phe Asn Gly Ser Ile Pro Asp Thr Val Ala Ser Leu Pro Glu Leu
305 310 315 320

Gln Gln Leu Asp Leu Ser Ser Asn Thr Phe Ser Gly Thr Ile Pro Ser
325 330 335

Ser Leu Cys Gln Asp Pro Asn Ser Lys Leu His Leu Leu Tyr Leu Gln
340 345 350

Asn Asn Tyr Leu Thr Gly Gly Ile Pro Asp Ala Val Ser Asn Cys Thr
355 360 365

Ser Leu Val Ser Leu Asp Leu Ser Leu Asn Tyr Ile Asn Gly Ser Ile
370 375 380

Pro Ala Ser Leu Gly Asp Leu Gly Asn Leu Gln Asp Leu Ile Leu Trp
385 390 395 400

Gln Asn Glu Leu Glu Gly Glu Ile Pro Ala Ser Leu Ser Arg Ile Gln
405 410 415

Gly Leu Glu His Leu Ile Leu Asp Tyr Asn Gly Leu Thr Gly Ser Ile
420 425 430

Pro Pro Glu Leu Ala Lys Cys Thr Lys Leu Asn Trp Ile Ser Leu Ala
436 440 445

Ser Asn Arg Leu Ser Gly Pro Ile Pro Ser Trp Leu Gly Lys Leu Ser
450 455 460

Tyr Leu Ala Ile Leu Lys Leu Ser Asn Asn Ser Phe Ser Gly Pro Ile

465 470 475 480

Pro Pro Glu Leu Gly Asp Cys Gln Ser Leu Val Trp Leu Asp Leu Asn
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Ser Asn Gln Leu Asn Gly Ser Ile Pro Lys Glu Leu Ala Lys Gln Ser
500 505 510

Gly Lys Met Asn Val Gly Leu Ile Val Gly Arg Pro Tyr Val Tyr Leu
515 520 525

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530 535 540

Glu Phe Thr Ser Ile Arg Pro Asp Asp Leu Ser Arg Met Pro Ser Lys
545 550 555 560

Lys Leu Cys Asn Phe Thr Arg Met Tyr Val Gly Ser Thr Glu Tyr Thr
565 570 575

Phe Asn Lys Asn Gly Ser Met Ile Phe Leu Asp Leu Ser Tyr Asn Gln
580 585 590

Leu Asp Ser Ala Ile Pro Gly Glu Leu Gly Asp Met Phe Tyr Leu Met
595 600 605

Ile Met Asn Leu Gly His Asn Leu Leu Ser Gly Thr Ile Pro Ser Arg
610 615 620

Leu Ala Glu Ala Lys Lys Leu Ala Val Leu Asp Leu Ser Tyr Asn Gln
625 630 635 640

Leu Glu Gly Pro Ile Pro Asn Ser Phe Ser Ala Leu Ser Leu Ser Glu
645 650 655

Ile Asn Leu Ser Asn Asn Gln Leu Asn Gly Thr Ile Pro Glu Leu Gly
660 665 670

Ser Leu Ala Thr Phe Pro Lys Ser Gln Tyr Glu Asn Asn Thr Gly Leu
675 680 685

Cys Gly Phe Pro Leu Pro Pro Cys Asp His Ser Ser Pro Arg Ser Ser
690 695 700

Asn Asp His Gln Ser His Arg Arg Gln Ala Ser Met Ala Ser Ser Ile
705 710 715 720

Ala Met Gly Leu Leu Phe Ser Leu Phe Cys Ile Ile Val Ile Ile Ile
725 730 735

Ala Ile Gly Ser Lys Arg Arg Arg Leu Lys Asn Glu Glu Ala Ser Thr
740 745 750

Ser Arg Asp Ile Tyr Ile Asp Ser Arg Ser His Ser Ala Thr Met Asn
755 760 765

Ser Asp Trp Arg Gln Asn Leu Ser Gly Thr Asn Leu Leu Ser Ile Asn
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Val Glu Ala Thr Asn Gly Phe His Ile Ala Cys Gln Ile Gly Ser Gly
805 810 815

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820 825 830

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Thr Ala Glu Met Glu Thr Ile Gly Lys Ile Lys His Arg Asn Leu Val
850 855 860

Pro Leu Leu Gly Tyr Cys Lys Ala Gly Glu Glu Arg Leu Leu Val Tyr
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Asp Tyr Met Lys Phe Gly Ser Leu Glu Asp Val Leu His Asp Arg Lys

885 890 895

Lys Ile Gly Lys Lys Leu Asn Trp Glu Ala Arg Arg Lys Ile Ala Val
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915 920 925

Ile Ile His Arg Asp Met Lys Ser Ser Asn Val Leu Ile Asp Glu Gln
930 935 940

Leu Glu Ala Arg Val Ser Asp Phe Gly Met Ala Arg Leu Met Ser Val
945 950 955 960

Val Asp Thr His Leu Ser Val Ser Thr Leu Ala Gly Thr Pro Gly Tyr
965 970 975

Val Pro Pro Glu Tyr Tyr Gln Ser Phe Arg Cys Thr Thr Lys Gly Asp
980 985 990

Val Tyr Ser Tyr Gly Val Val Leu Leu Glu Leu Leu Thr Gly Lys Pro
995 1000 1005

Pro Thr Asp Ser Ala Asp Phe Gly Glu Asp Asn Asn Leu Val Gly Trp
1010 1015 1020

Val Lys Gln His Thr Lys Leu Lys Ile Thr Asp Val Phe Asp Pro Glu
1025 1030 1035 1040

Leu Leu Lys Glu Asp Pro Ser Val Glu Leu Glu Leu Leu Glu His Leu
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Lys Ile Ala Cys Ala Cys Leu Asp Asp Arg Pro Ser Arg Arg Pro Thr
1060 1065 1070

Met Leu Lys Val Met Ala Met Phe Lys Glu Ile Gln Ala Gly Ser Thr
1075 1080 1085

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:an artificially synthesized primer sequence

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<211> 19

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:an artificially synthesized primer sequence

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Claims

1. A DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2.

2. The DNA of claim 1, wherein the DNA is a cDNA or a genomic DNA.

3. The DNA of claim 1, wherein the DNA comprises a coding region of the nucleotide sequence of SEQ ID NO: 1 or 3.

4. A DNA encoding a protein which has 55% or more homology to the amino acid sequence of SEQ ID NO: 2 and which is functionally equivalent to a protein comprising the amino acid sequence of SEQ ID NO: 2, said DNA being selected from the group consisting of

   (a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2 in which one or more amino acids are substituted, deleted, added, and/or inserted; and
   (b) a DNA hybridizing under stringent conditions with a DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3.

5. The DNA of claim 4, wherein the DNA encodes a protein having a function selected from the group consisting of a function of increasing brassinosteroid sensitivity in a plant, a function of inducing elongation of internode cells of a stem of a plant, a function of positioning microtubules perpendicular to the direction of elongation in an internode of a stem of a plant, a function of suppressing elongation of an internode of a neck of a plant, and a function of increasing inclination of a lamina of a plant.

6. The DNA of claim 4 or 5, wherein the DNA is derived from a monocotyledonous plant.

7. The DNA of claim 6, wherein the DNA is derived from a plant of the *Gramineae* family.

8. A DNA encoding an antisense RNA complementary to a transcript of the DNA of any one of claims 1 to 7.

9. A DNA encoding an RNA having ribozyme activity which specifically cleaves a transcript of the DNA of any one of claims 1 to 7.

10. A DNA which encodes an RNA repressing expression of the DNA of any one of claims 1 to 7 due to co-suppression when expressed in a plant cell and which has 90% or more homology to the DNA of any one of claims 1 to 7.

11. A DNA which encodes a protein having a dominant negative phenotype to that of a protein encoded by the DNA of any one of claims 1 to 7.

12. A vector which comprises the DNA of any one of claims 1 to 7.

13. A transformed cell which comprises the DNA of any one of claims 1 to 7 or the vector of claim 12.

14. A protein encoded by the DNA of any one of claims 1 to 7.

15. A method for producing the protein of claim 14, the method comprising the steps of culturing the transformed cell of claim 13 and recovering an expressed protein from said transformed cell or a culture supernatant thereof.

16. A vector comprising the DNA of any one of claims 8 to 11.

17. A transformed plant cell comprising the DNA of any one of claims 1 to 11 or the vector of claim 12 or 16.

18. A transformed plant comprising the transformed plant cell of claim 17.

19. A transformed plant which is a progeny or a clone of the transformed plant of claim 18.

20. A breeding material of the transformed plant of claim 18 or 19.

21. An antibody which binds to the protein of claim 14.

FIG. 1

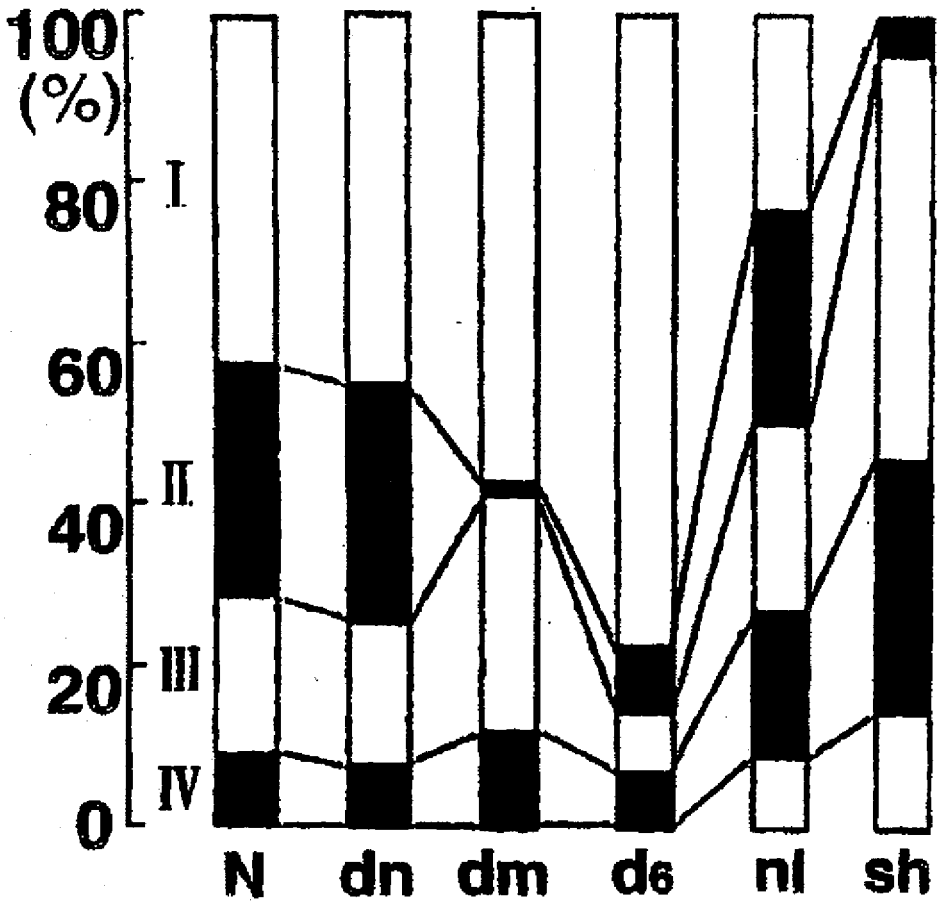

FIG. 2

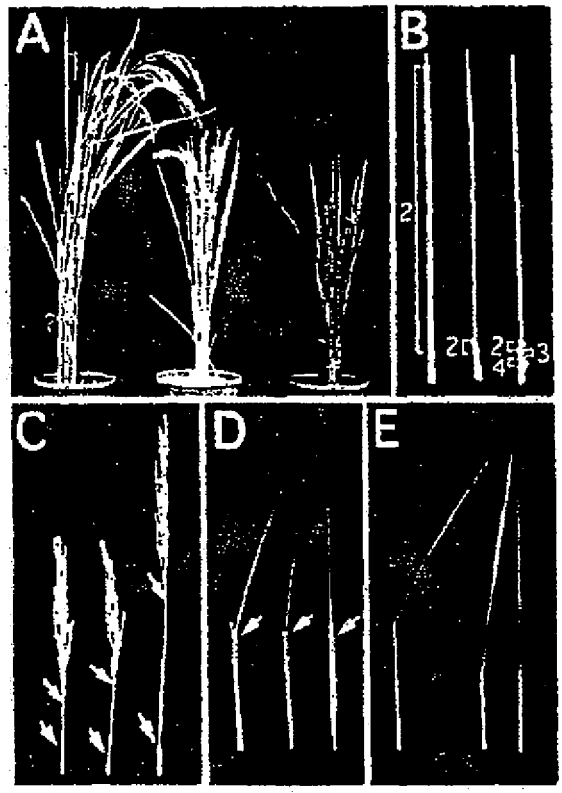

FIG. 3

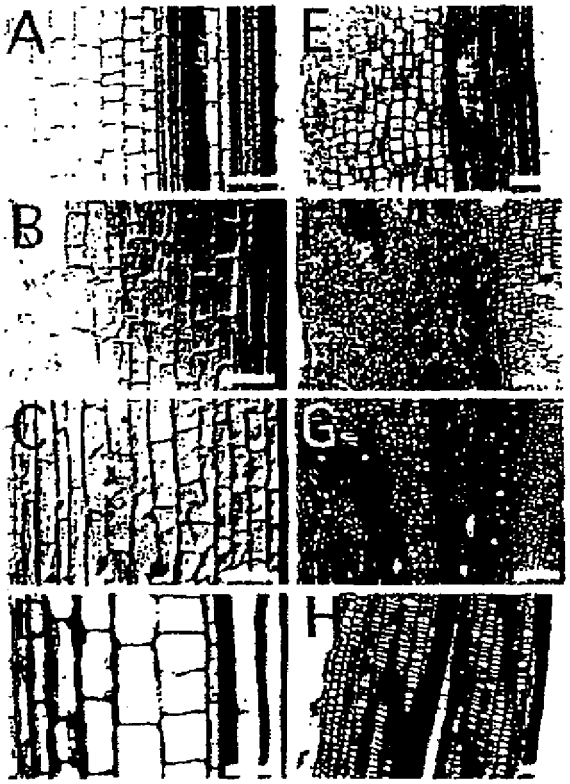

FIG. 4

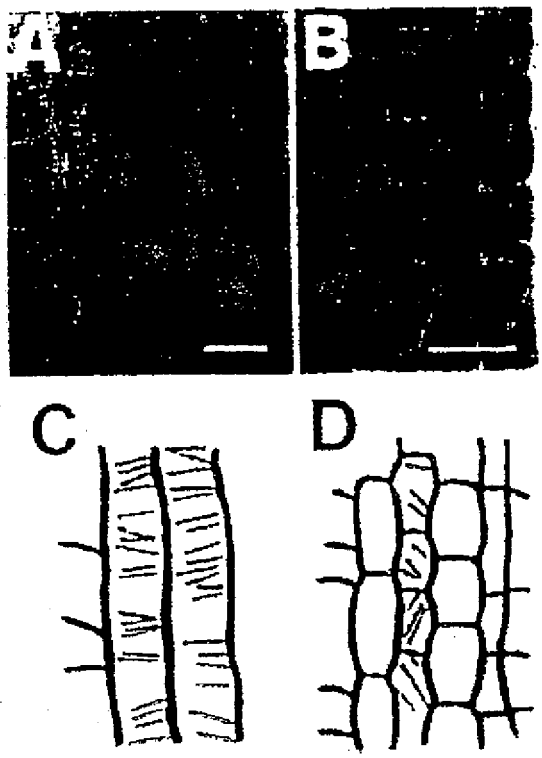

FIG. 5

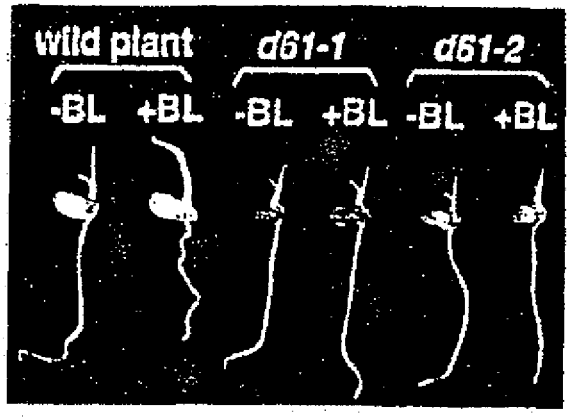

FIG. 6

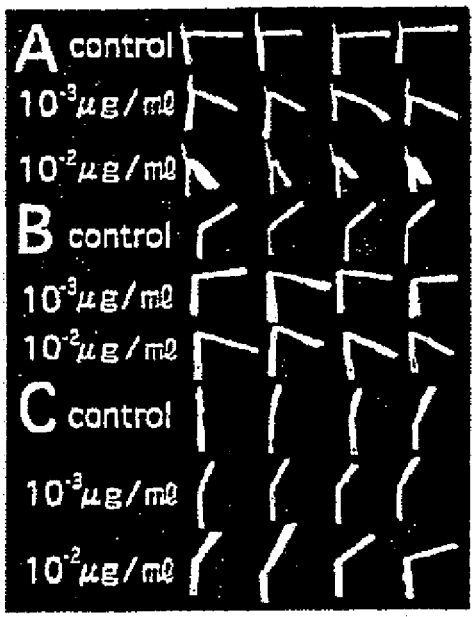
A control
10-3μg/mℓ
10-2μg/mℓ
B control
10-3μg/mℓ
10-2μg/mℓ
C control
10-3μg/mℓ
10-2μg/mℓ

FIG. 7

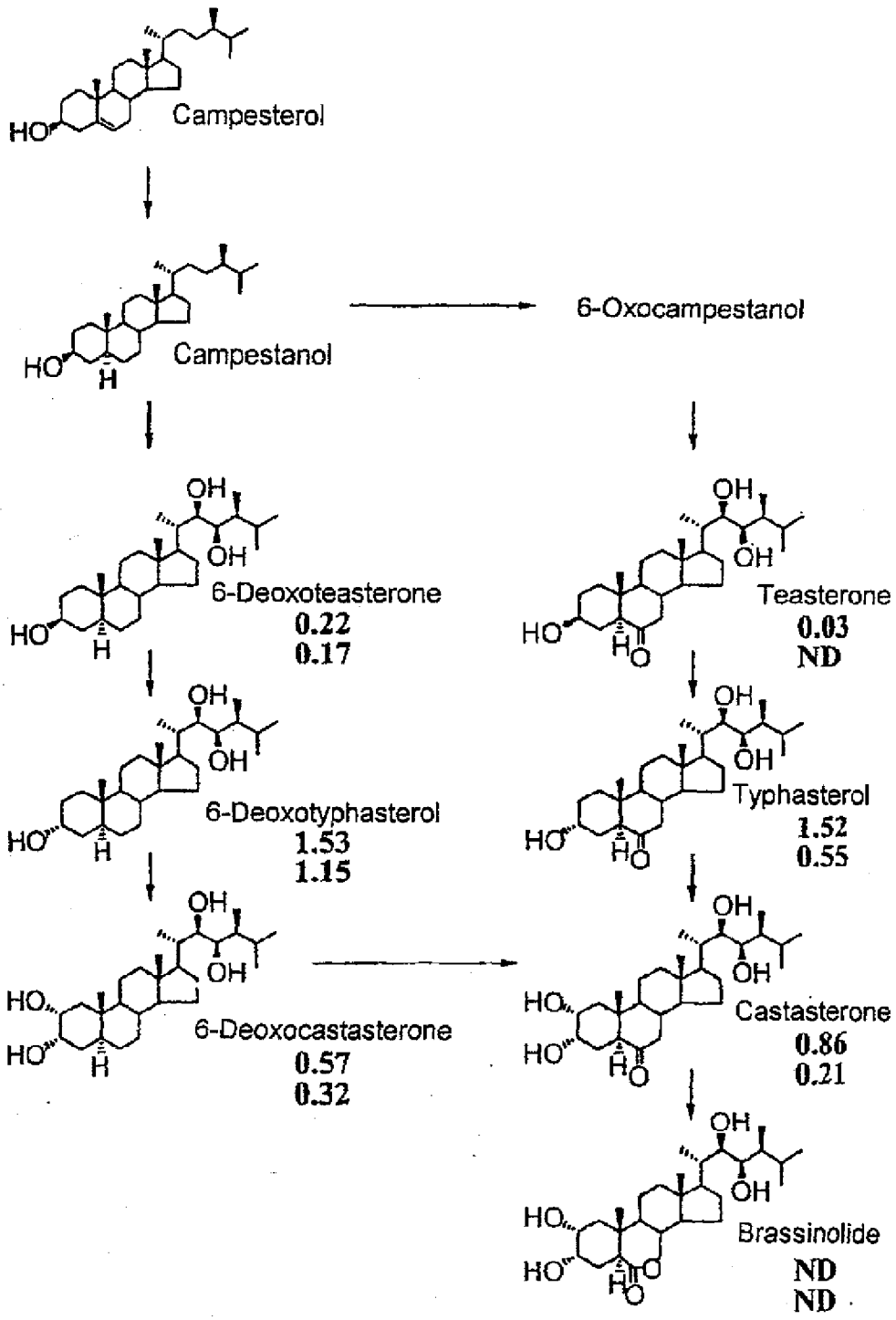
Campesterol
Campestanol
6-Oxocampestanol
6-Deoxoteasterone
0.22
0.17
Teasterone
0.03
ND
6-Deoxotyphasterol
1.53
1.15
Typhasterol
1.52
0.55
6-Deoxocastasterone
0.57
0.32
Castasterone
0.86
0.21
Brassinolide
ND
ND

FIG. 8

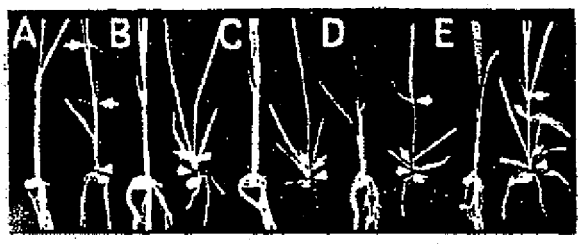

FIG. 9

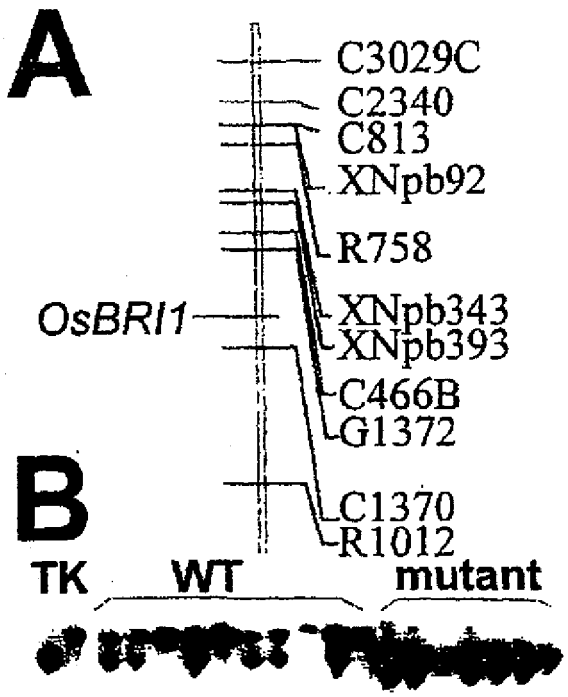
A
C3029C
C2340
C813
XNpb92
R758
OsBRI1
XNpb343
XNpb393
C466B
G1372
B
C1370
R1012
TK
WT
mutant

FIG. 10

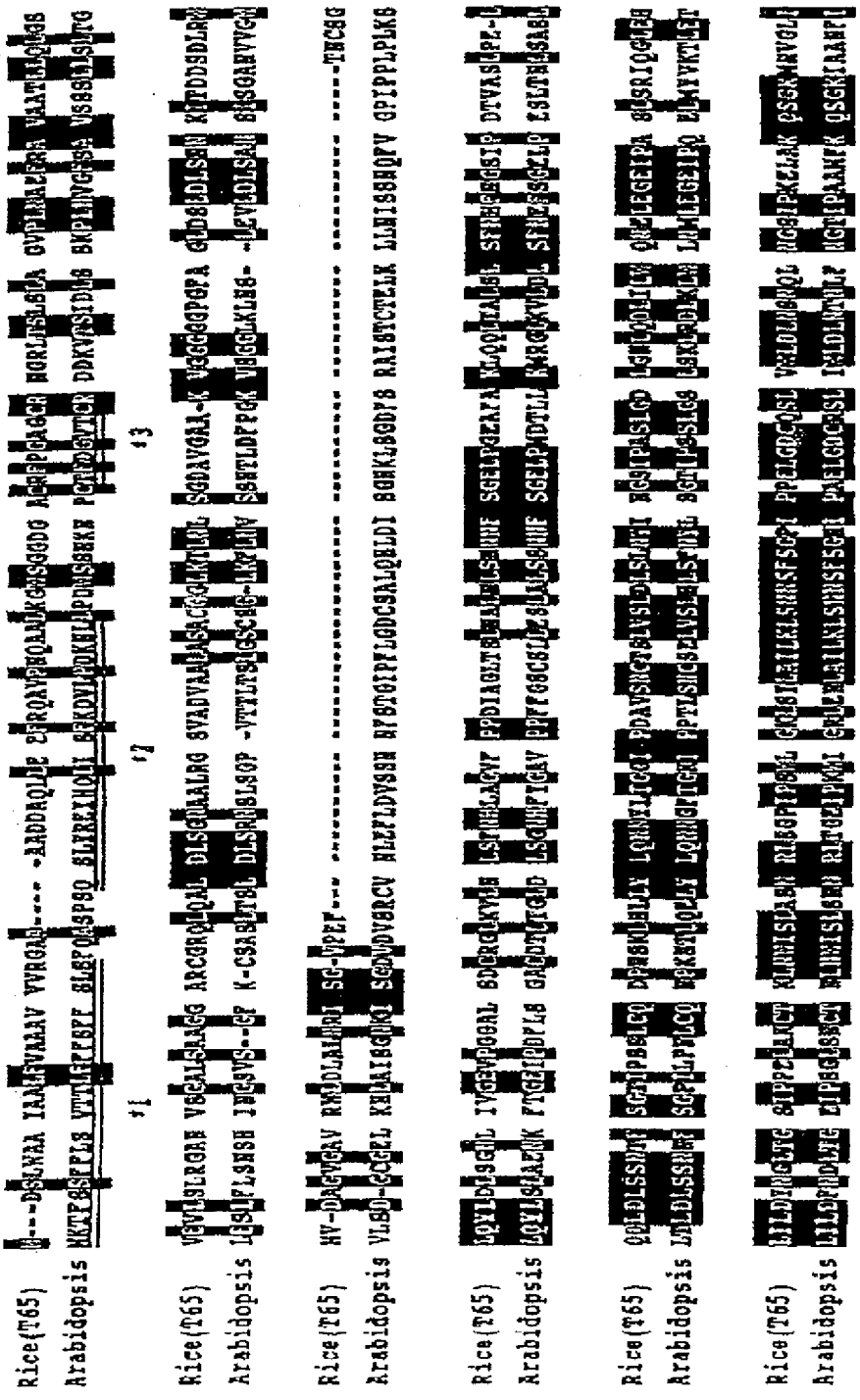

FIG. 11

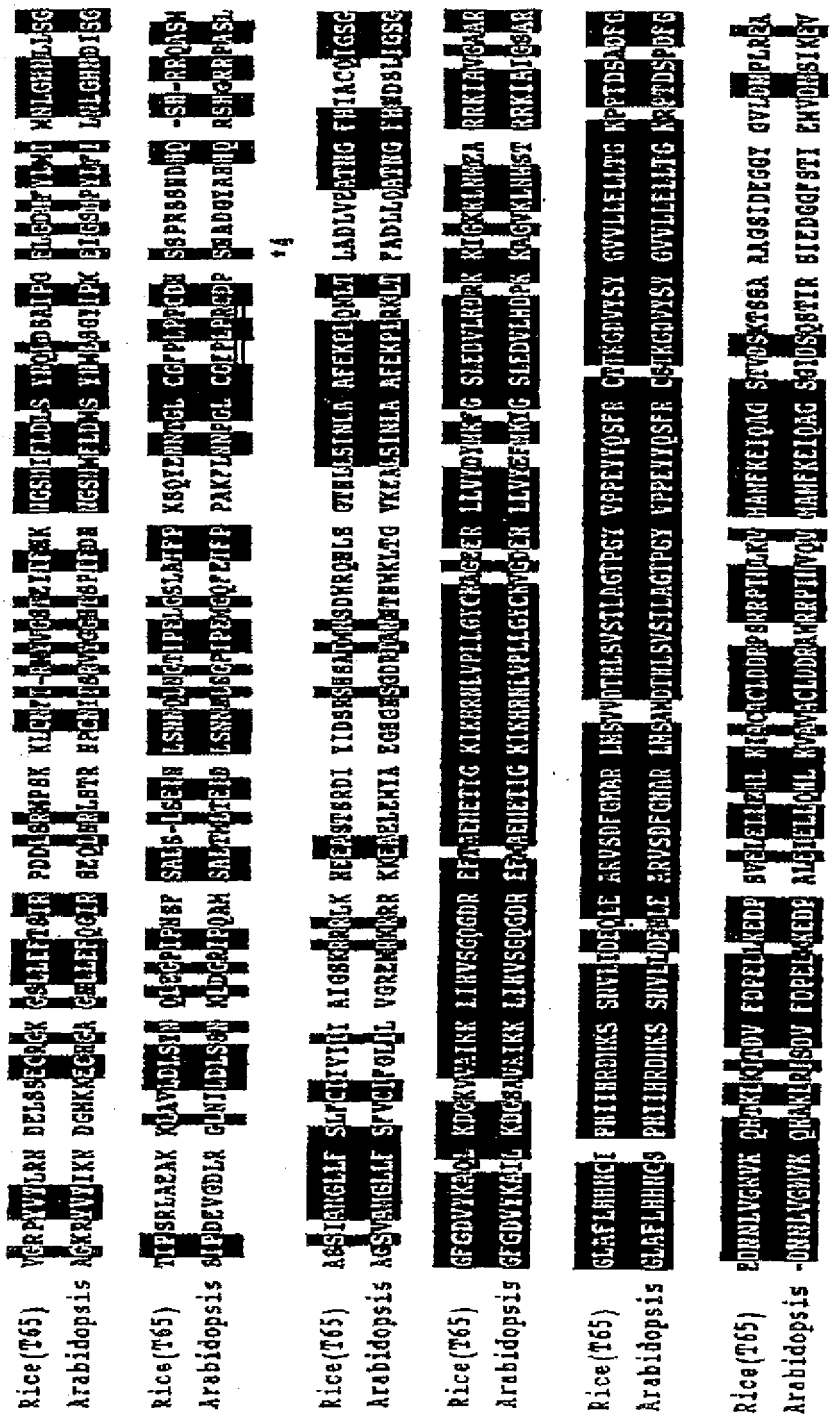

FIG. 12

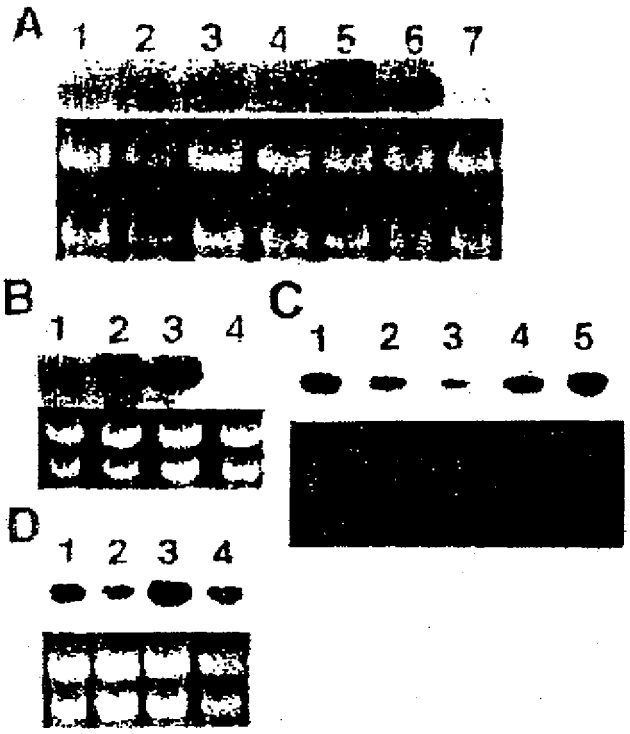

FIG. 13

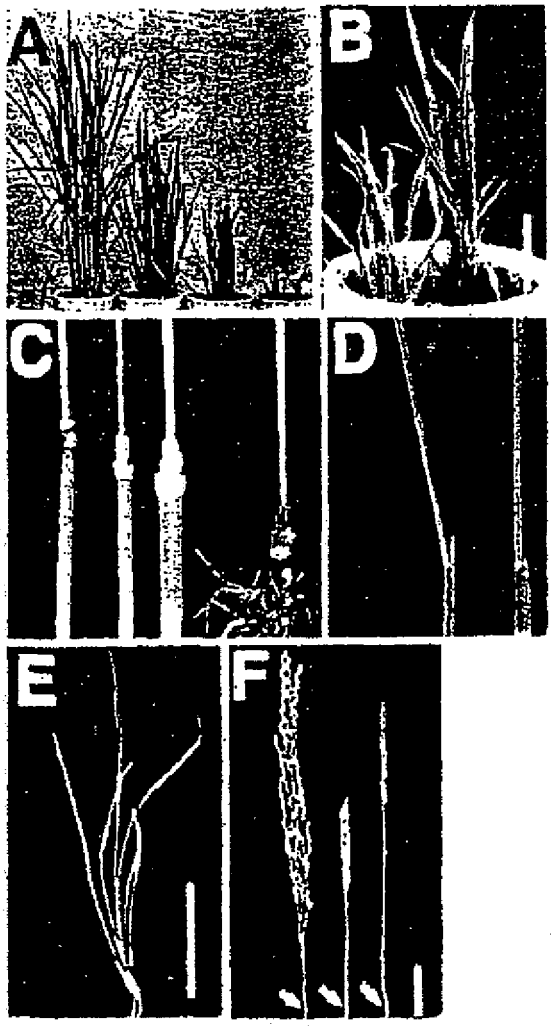

FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP01/02770

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C12N15/29, C12N15/63, C12N5/04, C07K14/415, C07K16/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C12N15/29, C12N15/63, C12N5/04, C07K14/415, C07K16/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
SwissProt/PIR/Genbank/Geneseq, MEDLINE (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Jianming Li., et al., "A Putative Leucine-Rich Repeat Receptor Kinase Involved in Brassinosteroid Signal Transduction", Cell, (1997), pages 184 to 193	1-21
A	Yano M, et al., "Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS", Plant Cell (December, 2000), Vol.12, No.12, pages 2473 to 2484	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 May, 2001 (30.05.01)	12 June, 2001 (12.06.01)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)